



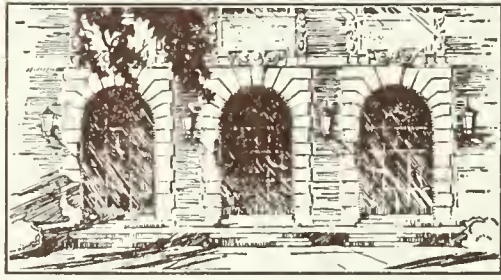
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ASPECTS OF CHAMBER FORMATION BY
ARCHIAS ANGULATUS, A FORAMINIFER

BY

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B.S., University of Illinois, 1961

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THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY
SUPERVISION BY DONALD STANLEY MARSZALEK

ENTITLED ASPECTS OF CHAMBER FORMATION BY

ARCHIAS ANGULATUS, A FORAMINIFER

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INTRODUCTION

Foraminifera constitute one of the largest groups of protozoa; more than 30,000 fossil and about 5,000 living species have been described. The organism is characterized by a shell or "test" which in most species is readily fossilized. The test may be composed entirely of secreted organic material as in Allogromia, of detritus bound in an organic matrix as in Bathysiphon, or of calcium carbonate of biogenic origin with lesser amounts of organic material as in Globigerina. Among foraminiferologists, those forms characterized by organic tests are considered "primitive", and forms with calcified tests are regarded as "advanced". Taxonomy within the group is based primarily on differences in test composition and microstructure and secondarily on differences in test morphology including chamber arrangement and apertural characteristics (Loeblich and Tappan, 1964.) So little work has been done on the living organism that a classification based on biological differences and affinities among foraminifers is virtually nonexistent.

The test is of immense economic importance to the petroleum industry as fossil foraminiferal tests are among the most important stratigraphic and environmental indicators for petroleum geologists. In spite of the voluminous amount of literature that exists on the foraminifera, most deals with the stratigraphic distribution of empty tests. Very little is known of relations of the test and the living organism. It is the purpose of this paper to examine the process of chamber formation in Archias angulatus, a shallow water benthic foraminifer which secretes a

multichambered test composed of calcite and organic material (figs. 1 and 2).

Foraminifers which secrete tests of calcium carbonate do so by the addition of new chambers at irregular intervals during the life cycle. Little is known of the chamber forming process. Several incomplete descriptions occur in the literature as secondary observations made while studying other aspects of foraminiferal behavior or structure.

Myers (1940) described chamber formation by Discorbis patelliformis in a paper dealing with reproduction, and later (1943) by Tretomphalus bulloides while studying the ecology of this "pelagic" foraminifer. He observed that D. patelliformis constructed a growth cyst prior to chamber formation; the cyst was formed from debris gathered by the pseudopods. A mass of cytoplasm then emerged from the test and assumed the form of the new chamber. Small bright points representing calcium carbonate then appeared on the surface. The calcified areas increased in size until the entire chamber became calcified. The process of chamber formation in D. patelliformis required about 12 hours.

Jepps (1942) observed chamber formation in Elphidium crispum. Following formation of a growth cyst, the foraminifer secreted an organic chamber which is gradually calcified. Jepps noted that pseudopods involved in membrane secretion were distinct from normal feeding pseudopods; those engaged in chamber formation were shorter, more rigid, and more closely spaced.

Le Calvez (1953) gave a general description of chamber formation by Discorbis bertheloti in the "Traite de Zoologie". As in many other species, a growth cyst was formed by the pseudopods from detritus. The pseudopods retracted away from the cyst and an organic membrane was secreted. Calcification began at numerous points on the membrane surface and continued until the entire chamber was mineralized. Le Calvez recognized the importance of pseudopods during chamber formation. He also noted increased cytoplasmic streaming within the test during chamber building.

Arnold (1964), in a biological study of Spiroloculina hyalina, observed numerous partially calcified chambers of that foraminifer. He concluded that in Spiroloculina, calcification occurred within an organic matrix rather than on an organic membrane, and that mineralization began at the cytoplasm-matrix interface. Calcium carbonate deposition began at points which gradually increased in size and coalesced until the entire matrix had calcified. He noted that mineralization occurred in waves rather than continuously.

Angell (1967b) investigated chamber formation by Rosalina floridana. His comprehensive work was based on electron microscopy of thin sections as well as on light microscopy. Angell recognized several types of cytoplasm associated with chamber formation. He noted formation of a growth cyst followed by secretion of an anlage which served as a template for the secretion of an organic membrane. The pseudopods and adjacent cytoplasm contained much fibrillar material within vesicles at this stage.

Angell's work represents an initial step toward understanding the process of chamber formation in foraminifers, and is representative of the Discorbidae. The variety of wall structures encountered in foraminifers necessitates variation in the chamber forming process. Similar studies are needed of all the major groups before an understanding of test formation and calcification in the foraminifers is achieved.

MATERIALS AND METHODS

Several hundred specimens of A. angulatus were collected from two localities in Florida Bay. One site is at the northern extremity of Nine Mile Bank located 8.6 nautical miles south-southeast of Flamingo, Everglades National Park; the other is in Pine Channel between Big Pine Key and Middle Torch Key. Water depth at the collecting sites is about one meter at mean low tide.

The foraminifers are found attached to blades of Thalassia testudinum, a marine angiosperm which forms extensive "grass beds" in the area. At the time the collections were made, the Thalassia blades measured 15 to 25 cm. in length; each blade contained about 7 to 10 specimens of A. angulatus. Foraminiferal abundance varies throughout the year and is in part dependent on the availability of healthy grass beds. Local conditions such as storm frequency and contruction in the area affect the amount of suspended sediment in the water and influence the growth of Thalassia. Although A. angulatus is found throughout the year, it is most abundant during the summer months (Dr. W. Bock, personal communication). Collections were made during June, July, and August while a bloom of A. angulatus was occurring; it was the dominant species of foraminifera in the area. The specimens were gathered by towing a plankton net through the grass or by removing large quantities of the grass and dislodging the attached fauna by vigorously rubbing the blades together (Marszalek and Hay, 1968). The concentrate containing the

foraminifers was then decanted to remove most of the organic debris and transported in glass jars to the Institute of Marine Science at Miami. The habit of A. angulatus of attaching itself to vertical surfaces allows easy separation of this species from the concentrate by the following method. The concentrate is allowed to stand for several days in glass jars. Most of the specimens of A. angulatus migrate up the walls of the containers, from which they are easily removed in a semi-clean state with a large bore pipette. Further cleaning is accomplished with a fine brush and several changes of millipore (4.5μ pore dia.) filtered sea water.

Of the specimens collected, about 3% were found to be in various stages of chamber formation. A large supply of specimens actively forming chambers was needed for this investigation. The supply was insured by removing the last 2 or 3 rows of chambers from the test with a finely sharpened needle. The specimens were then cleaned and transferred to transparent, tightly sealing plastic containers. The containers were filled with sea water and a small amount of diatoms. After the organism had attached itself to the substrate, the container could be turned over and the specimen viewed in the light microscope. Within 3 to 4 days, about 60% of the prepared specimens began to form new chambers. Chamber formation induced by removal of a portion of the test appeared identical to that occurring in non-disturbed specimens.

Routine light microscopic observations were made with a Leitz stereomicroscope, and with a Leitz Ortholux microscope equipped with achromatic phase contrast objectives and a Leica 35 mm. camera.

Photomicrographs were made with Kodak Plus-X Pan film developed in Ethol ultra-fine-grain developer.

Specimens prepared for transmission electron microscopy were fixed in 5% glutaraldehyde in sea water buffered with sodium cacodylate to pH 7.2, followed by post fixation in 1% osmium tetroxide in sea water. After fixation, those specimens in fragile stages of chamber formation were embedded in a 1% agar solution (after Haller, G. de, et al, 1961). Normal specimens not engaged in chamber formation to be used as a control were decalcified in sea water lowered to pH 2.0 by the addition of concentrated hydrochloric acid. The specimens were then dehydrated in graded solutions of ethanol and embedded under vacuum in Epon 812 resin. Thin sections were cut on glass and diamond knives with a Reichert Um 2 ultramicrotome and stained with lead citrate (Reynolds, 1963) and 2% uranyl acetate (Watson, 1958). The prepared thin sections were routinely examined in a Phillips 100 B electron microscope operated at 40, 60, and 100 kV. Transmission electron micrographs were made with a JEM 7 electron microscope operated at 50 and 80 kV. Kodak contrast plate film was used and developed in Kodak D-19 developer.

Specimens utilized for scanning electron microscopy were prepared in several ways. All specimens were fixed in 5% glutaraldehyde in sea water (pH = 7.2). After fixation, some were cleaned by ultrasonic treatment for 15 sec. while in a sea water bath. Most of the debris which normally adheres to the surface of the test was removed at this time. The cleaning procedure was then repeated in millipore filtered sea water.

The prepared specimens were then subjected to various treatments, depending on what information was to be obtained in the SEM. Those specimens utilized for examination of the organic membranes lining the test were allowed to undergo total decalcification in sea water of pH 2.0, and were then fixed again in 5% glutaraldehyde in sea water. The second fixation was assumed necessary because portions of the organic test liner previously embedded in the calcite test were probably not exposed to the original fixation. The specimens were then washed several times in pure water, quick-frozen over liquid Nitrogen, and freeze-dried in a Pearse Tissue Dryer (Small and Marszalek, 1969; Marszalek and Small, 1969). Specimens intended for examination of wall structure were etched in acidic sea water lowered to pH = 2.0 with concentrated hydrochloric acid. All specimens were mounted whole or fractured on double stick cellophane tape, coated with evaporated Au-Pd and examined in the Cambridge Stereoscan scanning electron microscope operated at accelerating voltages of 10 and 20 kV. The scan images were photographed on Polaroid type 55 PN film.

All illustrations in this report are of Archias angulatus.

Plate 1

Figure 1 Archias angulatus. Adult specimen. The test surface is characterized by numerous pits or "pseudopores." The pits are superficial and do not perforate the test. Each chamber row has several internal partitions not visible in this micrograph.

Scanning electron micrograph. Fixed in 5% glutaraldehyde in sea water and freeze-dried. X 70.

2 Internal cytoplasm occupies most of the chambers except for the last 2 rows.

Scanning electron micrograph. Fixed, decalcified, post-fixed, and freeze-dried. X 75.



RESULTS

A. Light microscopic observations

The process of chamber formation in A. angulatus occurs in several distinct stages. Figures 3, 4, 5, 6, 7, 8, and 9 are drawings based on observations in the stereomicroscope of the process of chamber formation by numerous specimens, and on light micrographs.

Stage 1. Cyst formation

The first visible sign of chamber formation is the construction of a growth cyst. The formation of cyst during growth and reproduction is widespread among the foraminifera. In Archias, the pseudopods are extended as during normal feeding, and debris in the area is picked up and transported by the pseudopods toward the test (fig. 3). The transported debris collects at a distance (of about one-fifth the test diameter) from the apertural face of the test, forming an arc-like band. The band of debris is cemented to the substrate with a substance secreted by the pseudopods. Specimens used in this study were supplied with a small amount of algae introduced as food which also served as the cyst building material. The amount of algae was kept at a minimum so that the growth cyst would not interfere with observation of the chamber forming process. Specimens supplied with unlimited debris formed dense cysts which covered most of the test. No selectivity of cyst building materials was observed; specimens incorporated algae, minerals grains, and portions of fractured tests.

Although only a band of debris is illustrated in fig. 4, the cyst is in reality a sac-like structure. It extends as a sheath-like secretion from the band of debris along the substrate to a line of attachment along the bottom surface of the test, and from the band of debris through the sea water to a line of attachment along the upper surface of the test. The substance forming the sheath is derived from the pseudopods. It is transparent in transmitted light but can be observed by its surface sheen in properly oriented reflected light. The sheath is most easily observed by removing the foraminifer from the cyst with a fine probe. The disrupted cyst remains attached to the substrate with the upper and lower sheaths mostly intact. The cyst is durable enough to withstand the probing and crawling activities of various ciliates and other micro-organisms. It probably functions as a protective device to prevent disruption by foreign micro-organisms (which abound on Thalassia blades) of the delicate structures produced by the foraminifer during the earlier stages of chamber formation.

Stage 2. Pseudopod transformation.

The next stage in the process of chamber formation is illustrated in fig. 5. After the cyst is complete, the pseudopods are retracted within the cyst and assume the form shown in the drawing. At this stage, pseudopod form is characteristic of chamber formation and is very distinct from the pseudopodial net seen in foraminifers not engaged in chamber formation. All pseudopods are approximately $160\ \mu$ in

length and extend from the apertural face to the substrate. They are very closely spaced and occur in several layers so that the entire pseudopodial array has the appearance of a solid sheath of cytoplasm. Examination in the light microscope, however, reveals a multitude of separate parallel pathways of streaming cytoplasm. It is probable that the surface membranes of several adjacent pseudopods may coalesce without the loss of multiple pathways of bidirectionally streaming cytoplasm (Marszalek, 1969).

Another striking characteristic of pseudopods at this stage of chamber formation is the increased rate of bidirectional cytoplasmic streaming. The rate of movement of particles within the pseudopods as seen in phase contrast microscopy is 3 to 5 times that which occurs in normal feeding type pseudopods. The increased rate of cytoplasmic streaming manifests itself in the stereomicroscope and reflected light as a shimmering surface of cytoplasm with constantly changing reflective properties.

Stage 3. Anlage formation.

After the pseudopods have assumed the form illustrated in fig. 5, the anlage is formed. The anlage is a mass of cytoplasm, secreted by the pseudopods, which assumes the general shape of the chambers to be formed. Its probable function is to act as a substrate upon which the organic membrane of the test will be secreted. The first indication that the anlage is forming is a faint line indicating the presence of non-

pseudopodial cytoplasm, which is seen about half-way down the length of the streaming pseudopods (see fig. 6). The anlage cytoplasm gradually builds up from the line of demarcation to the apertural face. Secretion of the complete anlage takes 2 to 5 hours. At this stage during the process of chamber formation, the first indication of the general size and shape of the forming chamber is present. The anlage has a dull surface compared to the exposed portion of the streaming pseudopods. In reflected and transmitted light, it appears structureless.

Stage 4. Membrane formation.

Secretion of the organic membrane occurs after the anlage has been completed (fig. 7). As the organic portion of the chamber wall is secreted, the formerly dull surface of the anlage assumes a shiny appearance. The organic membrane is not merely a thin lining over the anlage but is a relatively massive structure which corresponds in thickness to the calcified chamber wall. An indication of its thickness can be seen in fig. 11 which shows the completed organic membrane. As the organic membrane is secreted, the chamber acquires its final shape. The surface of the membrane becomes very shiny and the pits or "pseudopores" are readily visible (figs. 7 and 11). At this stage no calcification can be observed, yet the chambers show the surface morphology of fully calcified chambers.

The exterior surface of the chambers seems to be formed first. Secretion of the internal chamber partitions (see fig. 10) occurs later

after the outer organic membrane is nearly completed. Several specimens have been observed with partially calcified outer walls and incompletely formed inner partitions, yet the older chambers usually have completed partitions except in areas where they were obviously broken away. It is probable that the organism is able to secrete the interior architecture of a chamber at some later time, after the outer walls have been calcified.

Several specimens ceased chamber building activity after the organic membrane was formed, and moved out of the growth cyst to resume normal feeding. The organic membrane is apparently rigid enough at this stage to allow the organism to resume some normal functions without the protection of the growth cyst. The usual case, however, is that the foraminifer remains in the growth cyst and continues to build the chamber.

Stage 5. Invasion by pigmented cytoplasm.

During the previous stages in the process of chamber formation, the new chamber has been colorless in contrast to the remainder of the test which is filled with vivid green cytoplasm. When the organic membrane appears complete and reflects the surface morphology of the older chambers, the inner cytoplasm containing the zoochlorellae invades the new chamber (see fig. 8). The invading cytoplasm is easily observed because of the color difference. The green cytoplasm enters the new chamber through the former apertures of the adjacent chamber row, and eventually fills the entire chamber interior. The invasion is complete within 1 to 2 hours. Pseudopod activity beyond the apertural face of the

new chamber may continue at a reduced rate or may cease entirely. With the vivid green cytoplasm filling all chambers, calcification occurs.

The shiny transparent organic chamber gradually assumes a milky white color. Complete calcification requires several days and most specimens move out of the growth cyst and resume normal feeding before it is complete.

Stage 6. Calcification.

Fig. 9 illustrates the foraminifer leaving the growth cyst to resume normal feeding. The pseudopods are now normal in form and are no longer characteristic of a specimen building a new chamber.

As stated earlier, the calcification process is gradual and requires several days for completion. No indication of how the calcite was being deposited could be seen with the light microscope; only the gradual change from transparent to opaque white was observed. During calcification, the chamber surface appeared clean indicating that it was not covered with a sheath of cytoplasm. Specimens of A. angulatus were able to build several successive rows of chambers at 2 to 3 day intervals, before calcification of the previously formed chambers had been completed. Tests with 3 and 4 rows of partially calcified chambers were not uncommon in the mass cultures. Observations on several species of foraminifers including Articulina, Peneroplis, and Sorites indicate that chamber formation is not continuous as is commonly believed. Increase in test size occurs at irregular intervals, usually with the addition of several chambers in a relatively short time followed by a period of stable test size.

Plate 2

Figure 3 A. angulatus initiating the process of chamber formation by collecting debris to form a growth cyst. Pseudopods collect and transport foreign particles which are then cemented to the substrate at a fixed distance from the apertural face. A portion of the growth cyst is seen in fig. 3.

Drawing based on light micrographs and visual observations of a living specimen. About X 50.

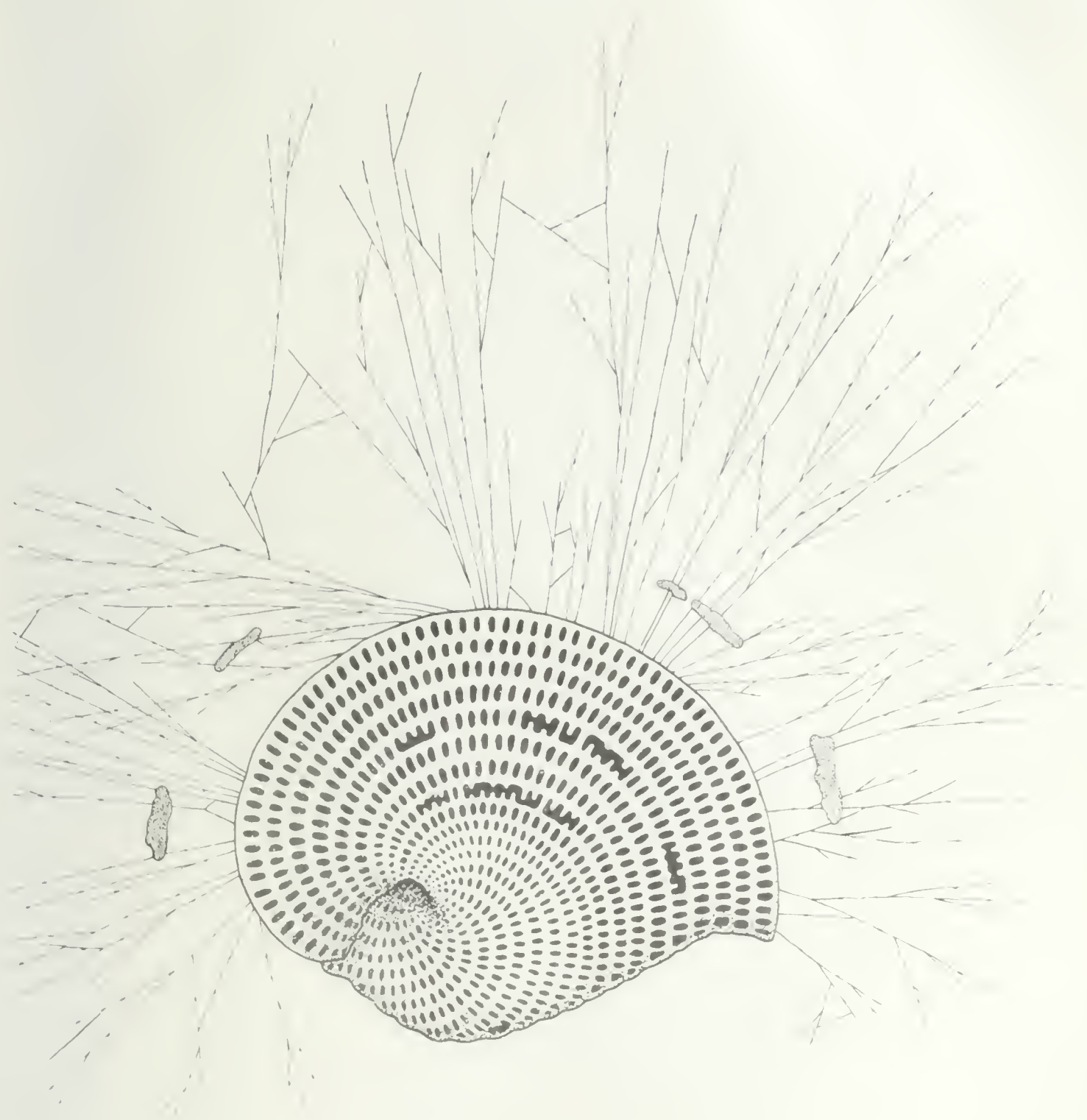


Plate 3

Figure 4 Stage 1. Cyst formation.

Formation of the growth cyst is complete. The cyst consists of a band of debris cemented to the substrate with organic secretions by the pseudopods. The cyst extends over most of the test as a transparent organic sheath. The line of attachment of the sheath is shown in the illustration. Drawing. About X 50.

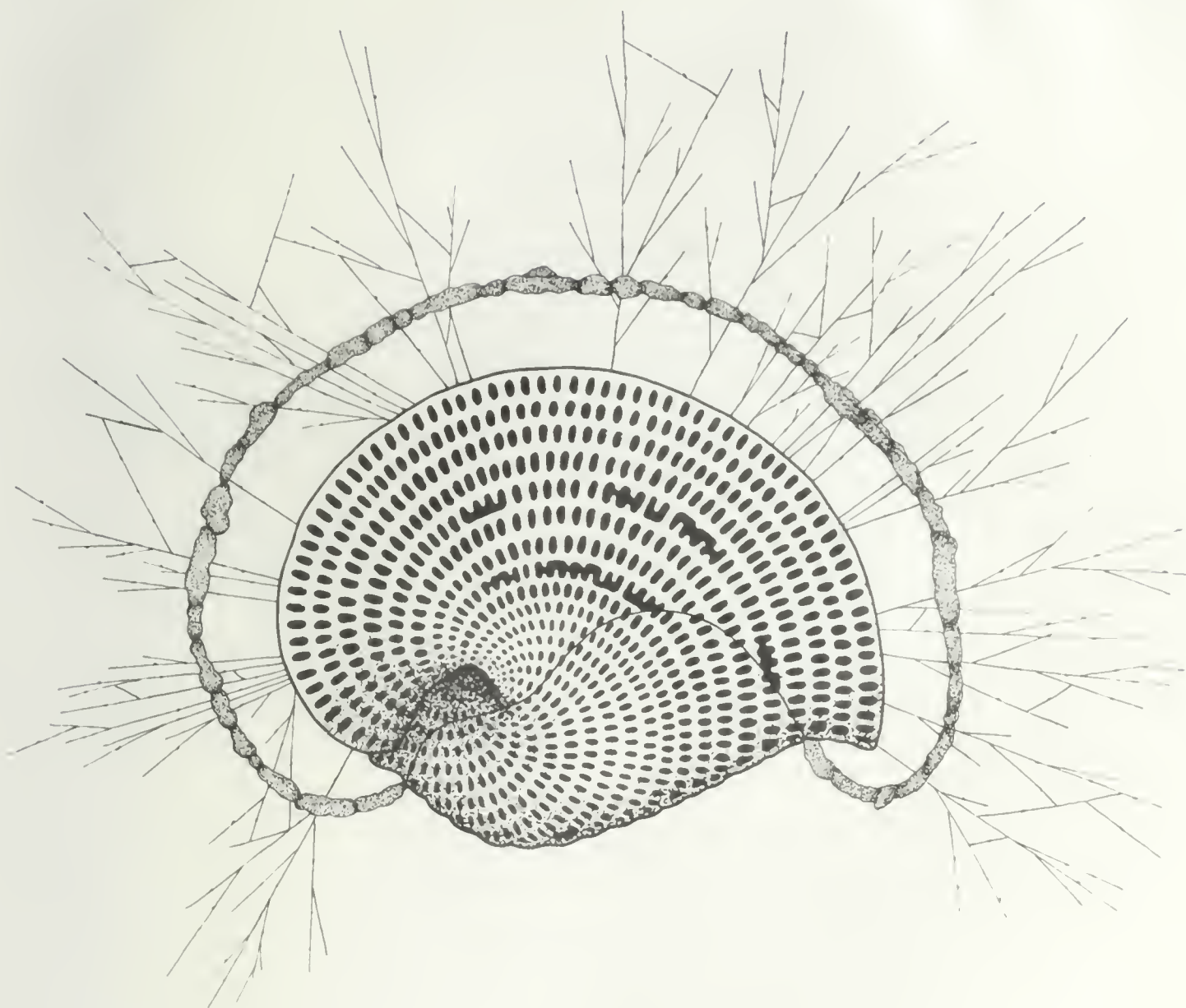


Plate 4

Figure 5 Stage 2. Pseudopod transformation.

Pseudopods assume the characteristic form shown in the illustration. This regular array has been seen only during chamber formation and is distinct from the normal pseudopodial net formed by foraminifera. Drawing. About X 50.

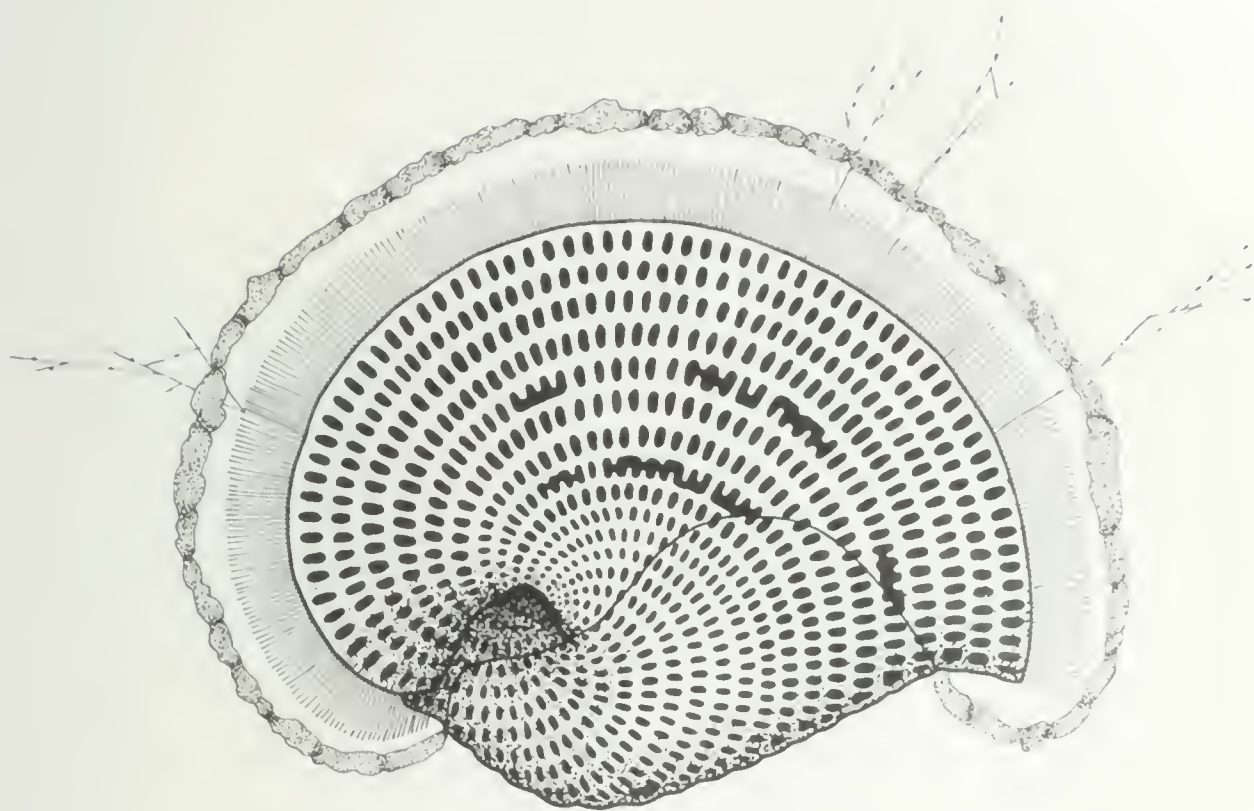


Plate 5

Figure 6 Stage 3. Anlage formation.

The anlage is a wedge-shaped mass of cytoplasm secreted by the pseudopods which functions as a substrate during secretion of the organic phase of the chamber. It establishes the general shape and size of the new chamber.

Drawing. About X 50.

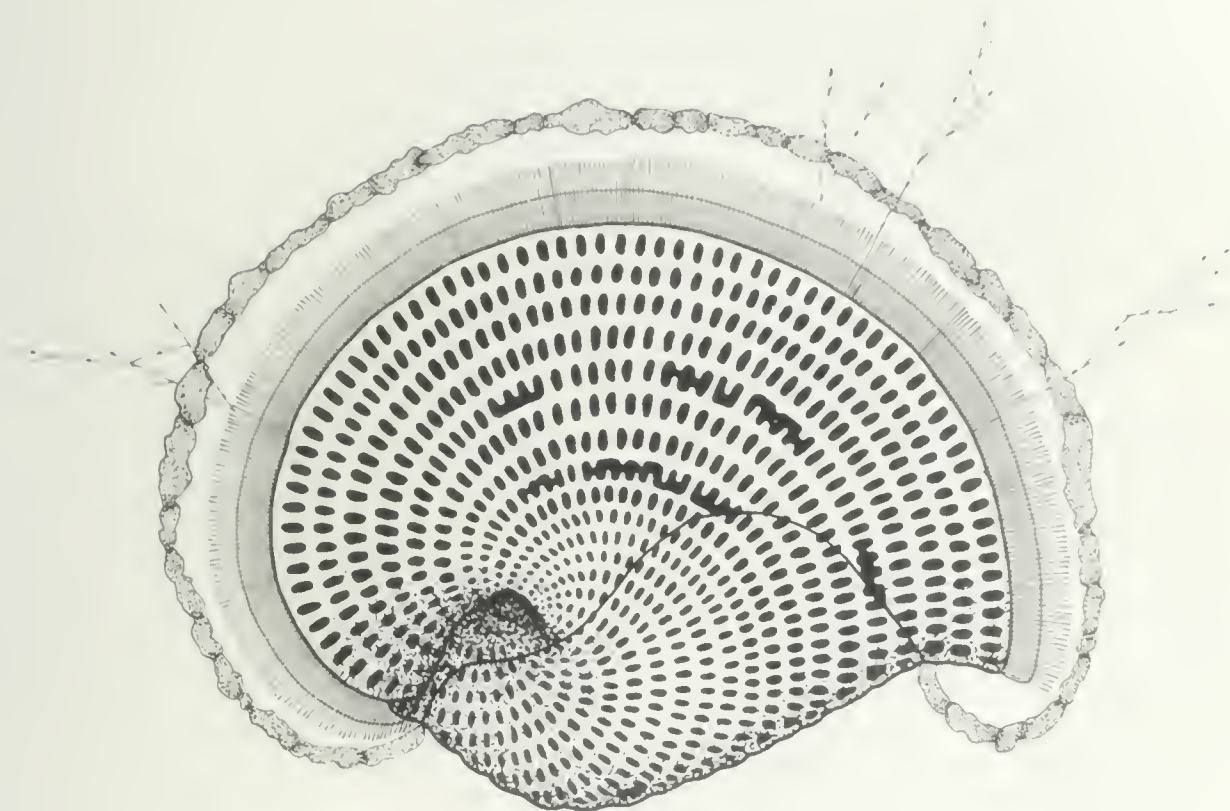


Plate 6

Figure 7 Stage 4. Membrane formation.

An organic membrane is secreted over the anlage. When completed as shown in the illustration, the organic membrane reflects the detail features of the calcified tests; note pits on surface. Drawing. About X 50.

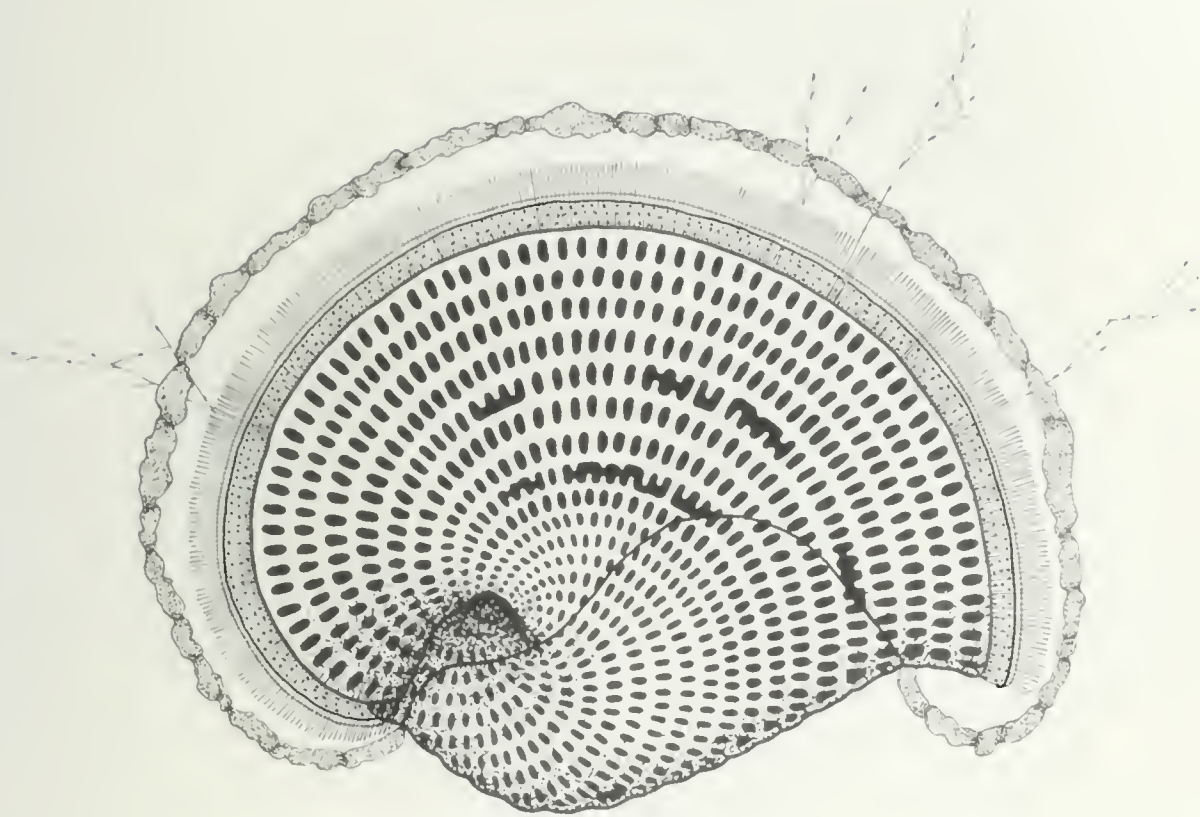


Plate 7

Figure 8 Stage 5. Invasion by pigmented cytoplasm. After the membrane is completely formed, cytoplasm containing zoochlorellae invades the organic membrane. The new chamber then assumes a vivid green color because of the numerous algal cells. Early stages of cytoplasmic invasion are illustrated. Drawing. About X 50.

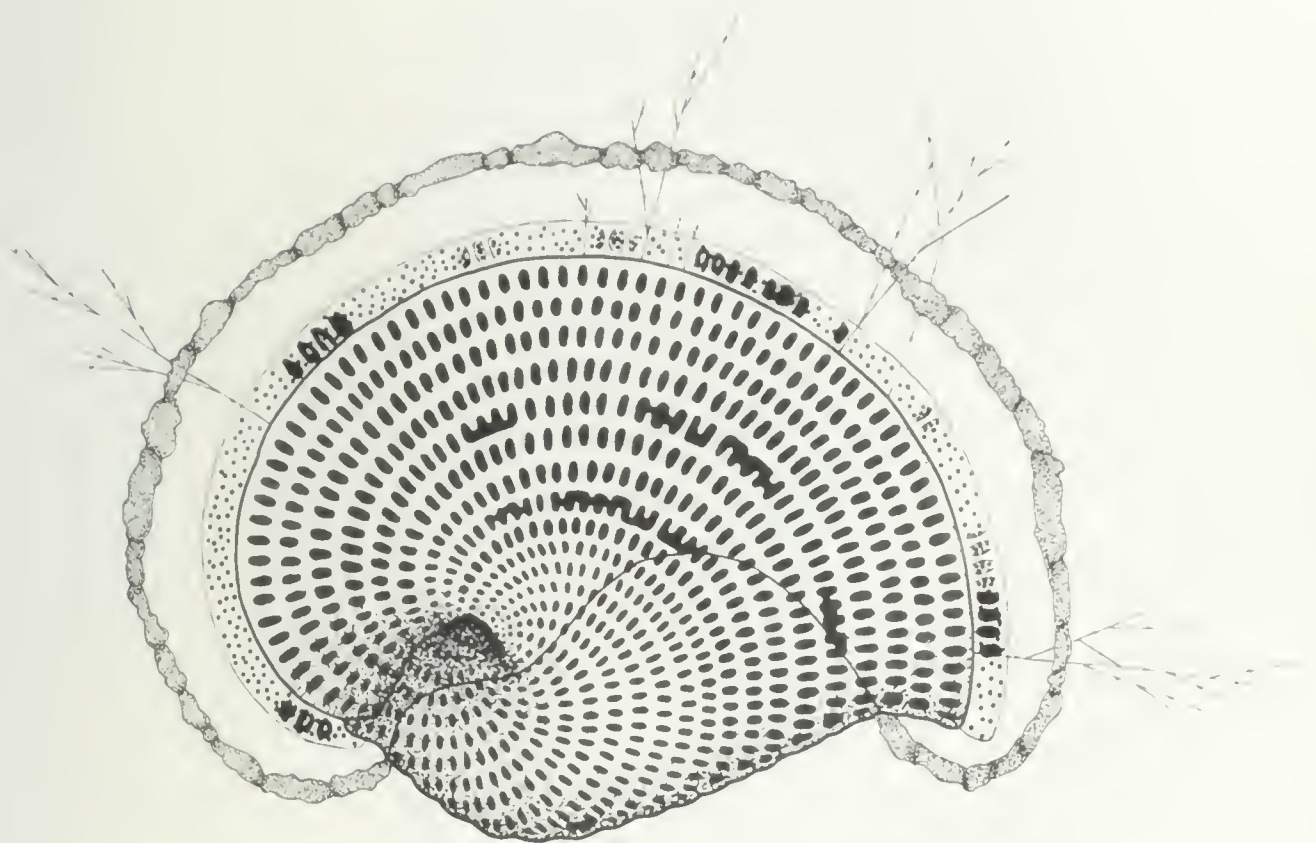


Plate 8

Figure 9 Stage 6. Calcification.

Calcification occurs after the pigmented cytoplasm invades the forming chamber. The calcification process usually requires 2 to 3 days. The organism has developed normal pseudopods and has broken out of the growth cyst as is shown in the illustration. Drawing. About X 35.

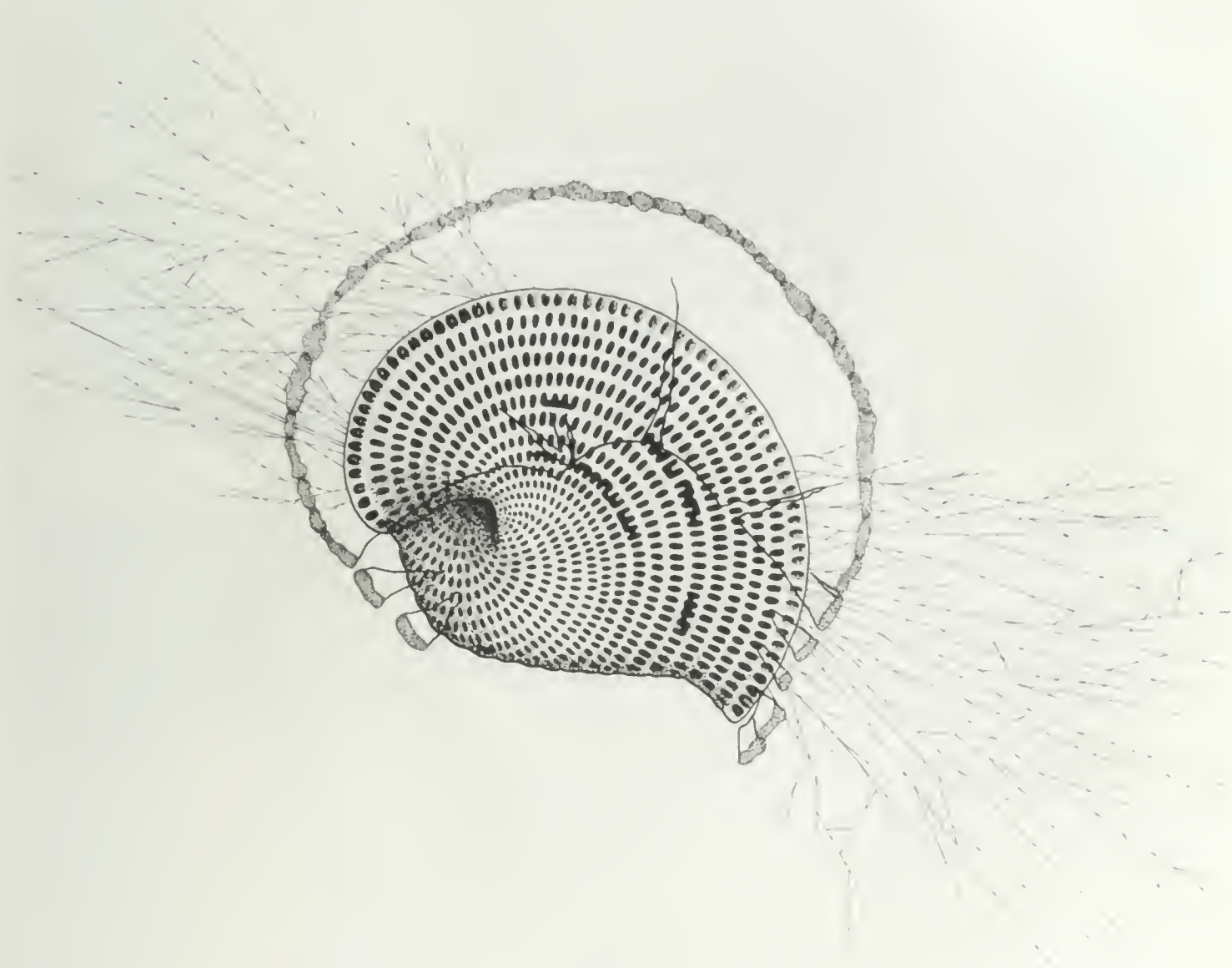


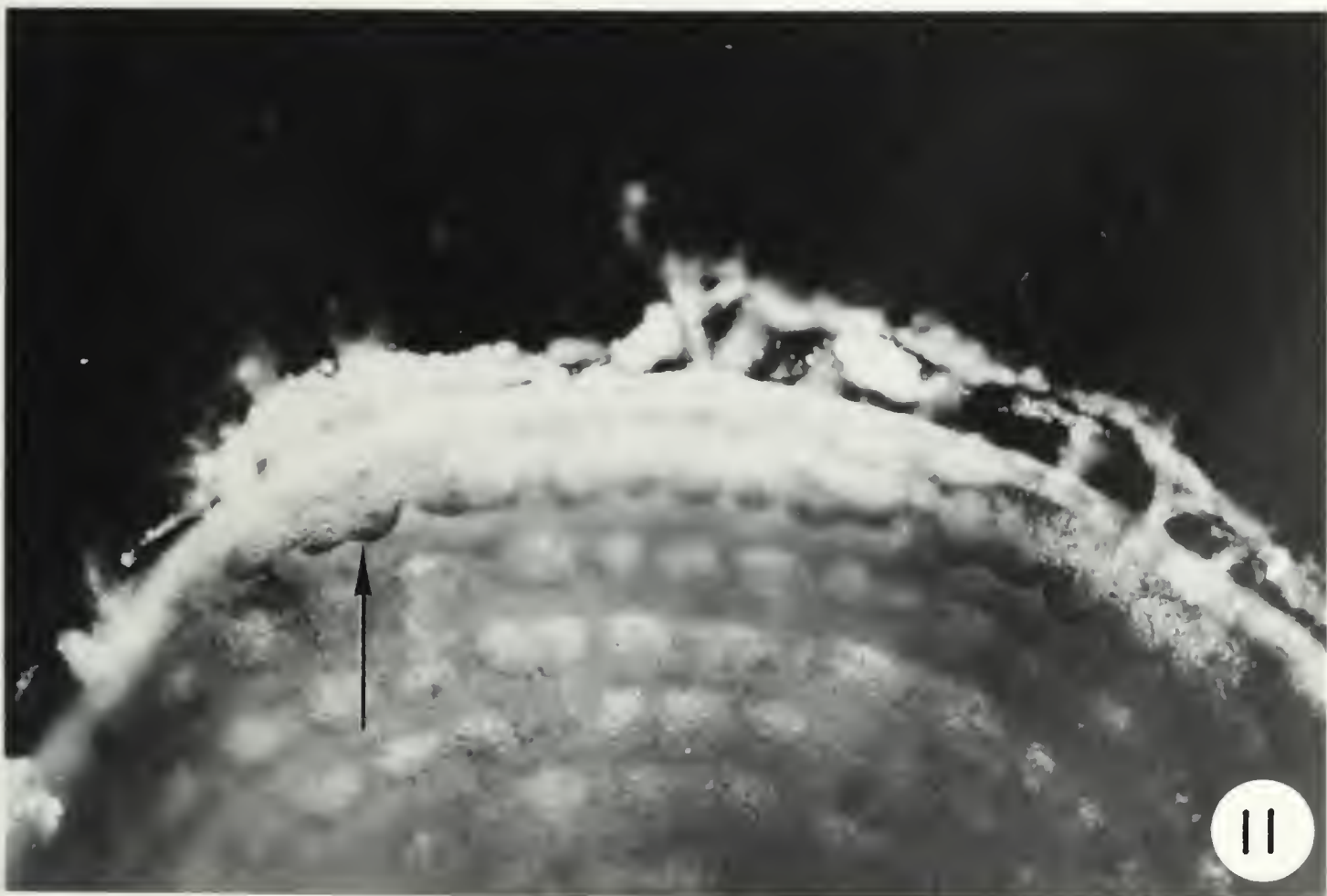
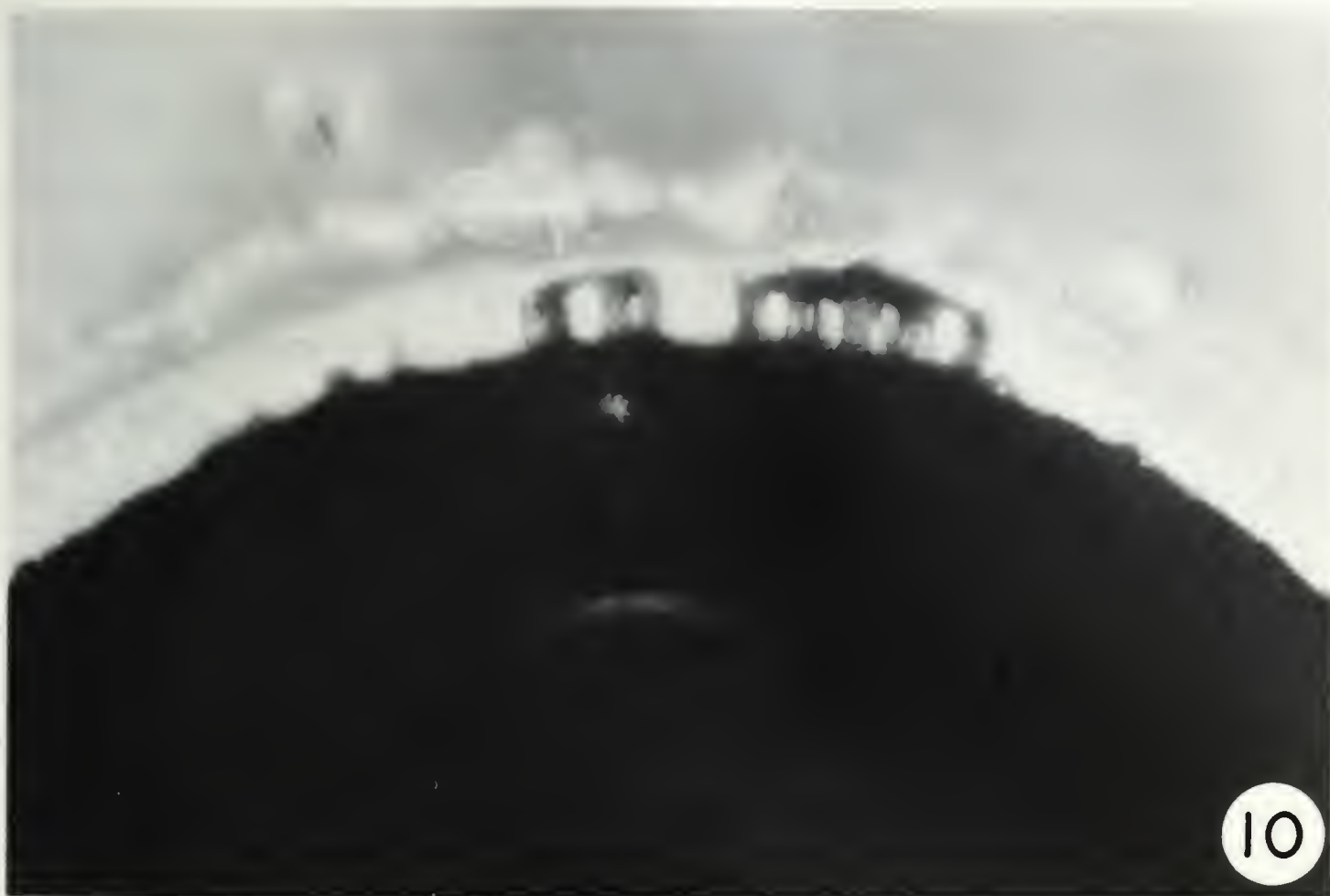
Plate 9

Figure 10 Several internal partitions remain after 3 chamber rows were removed to induce chamber formation. The growth cyst has been constructed and pseudopod transformation has occurred.

Light micrograph. Phase contrast. X 145.

- 11 Arrow indicates ragged chamber edge remaining after removal of several chamber rows. The organic membrane has completely formed; pits on membrane surface are visible above arrow.

Light micrograph. Dark field. X 145.



B. Electron microscopic observations.

Several specimens not forming chambers were studied for comparative purposes. The cytoplasm of specimens not engaged in chamber formation contains mitochondria, golgi, lipids, food vacuoles, vesicles, structures of unknown function, and zoochlorellae (figs. 12 and 13). Although relatively few published electron micrographs of foraminiferal cytoplasm exist, the normal cytoplasm of A. angulatus less the zoochlorellae resembles that which has been published (Wohlfarth-Bottermann, 1964; Hedley, et al., 1967; Angell, 1967), and that observed by the author in other species of foraminifers. The cytoplasm found in the last formed chamber row is usually less dense and is characterized by many vesicles. Zoochlorellae are not evenly distributed throughout the cytoplasm but are concentrated near the chamber walls, where they occur in groups of closely spaced cells (see fig. 14). No differences were noted between the normal cytoplasm of specimens not forming chambers and the cytoplasm of the inner chambers of specimens engaged in chamber formation. Modification of the cytoplasm occurs in the outermost chambers of specimens secreting new chambers; cytoplasm which occurs outside the test is also modified and does not contain zoochlorellae.

The secreted portion of the growth cyst is composed of a fibrous material (see figs. 15 and 16). An outer limiting membrane was not discernable in thin section. Occasional vesicles and mitochondria are also present but most of the volume of secreted material is fibrous in character. The average thickness of the cyst wall is about 2.5 μ . The

cyst wall is remarkably regular in form, appearing as a straight or curved line in thin section. The space between the cyst wall and the forming chambers appears mostly structureless but occasional groups of apparently free floating vesicles and a few mitochondria were observed (see fig. 15).

Before the chamber wall is secreted, a mass of cytoplasm is formed, presumably by modification of pseudopodial cytoplasm. This anlage cytoplasm assumes the general shape of the new chamber to be formed. It is characterized (fig. 17) by a mass of structureless vesicles interspersed with strands of membrane bound cytoplasmic units composed mainly of fibrous material and mitochondria. Fig. 17 represents the anlage cytoplasm at a stage in the process of chamber formation comparable to that illustrated in figs. 6 and 7.

Pseudopods extend through the anlage and secrete over it the organic component of the chamber walls. The pseudopods are characterized by structureless vesicles and membrane bound cytoplasmic units containing mitochondria, fibrillar material, and oval granules (see fig. 18). The oval granules characteristic of the pseudopods during chamber formation are probably compact units of fibrillar material produced by the cytoplasm within the test and transported by pseudopods to the site of chamber formation. In thin section the oval granules are about $1.2\ \mu$ long and $0.5\ \mu$ wide; they appear to have a structureless space between the dense core and the outer limiting membrane. Several oval granules have been observed in thin section which appear to be emptying their dense

core of compacted fibrillar material into the larger cytoplasmic unit (see arrow in fig. 20). The oval granules are apparently an efficient means of transporting the relatively large volume of fibrillar material necessary during chamber formation. The fibrillar material released from the oval granules is thought to coalesce and form the organic component of the new chamber wall.

At the stage of chamber formation illustrated in fig. 7, the non-calcified, organic chamber wall has a shiny appearance and reflects all the morphological features of the calcified test, including the surface pits or pseudopores (see fig. 11). The chamber interior is filled with numerous vesicles containing less dense fibrillar groundplasm as shown in fig. 19. These vesicles are thought to be spent, having given up their fibrillar contents to the organic chamber wall. Mitochondria are still present within some of the vesicles. At this time a membrane lies between the organic chamber wall and the cytoplasm of the chamber interior.

Cytoplasm from the inner chambers of the test then invades the newly formed chamber and calcification begins (figs. 21-23). The invading cytoplasm is less dense than normal but contains numerous zoochlorellae; these are dispersed throughout the cytoplasm but are concentrated in the outer portions of the new chamber, adjacent to the organic chamber wall. Mitochondria may occur free within the invading cytoplasm but more often they are still associated with membrane bound units of fibrillar material. The process of calcification is very gradual. The first appearance of calcite is marked by formation of crystallites in the chamber wall. The

mineralization continues until the newly formed chamber is completely calcified and resembles the older portion of the test. Little evidence for the process of calcification is apparent in the micrographs. The non-calcified organic test appeared structureless in thin section. With calcification, numerous needle-like structures appeared in the organic matrix (see fig. 20). The needles are randomly oriented and are thought to represent calcite crystallites. With continued calcification, the needle-like structures become more numerous and closely spaced. Specimens in advanced stages of calcification could not be sectioned adequately and were examined in the scanning electron microscope.

Figures 24, 25, and 26 show the general morphological features of the test of A. angulatus. The test surface is covered with numerous pits or pseudopores, which are not true pores but merely shallow depressions on the test surface. Although the pits are superficial features, their presence is reflected on the inner walls of the chambers as protrusions (see fig. 33). The multiple apertures of A. angulatus occur in roughly parallel rows along the outer margin of the chamber and remain full of cytoplasm during the later stages of chamber formation.

Specimens which were not ultrasonically cleaned before examination in the scanning electron microscope were covered with a thin organic sheath which extends over the entire test surface. The organic sheath is secreted by the pseudopods and probably serves to protect the calcite test from chemical dissolution. Specimens with the organic sheath intact required up to 60 hours for complete dissolution of the test in sea water

of pH 2.0. Specimens which were ultrasonically cleaned were completely decalcified under the same conditions in 2 to 4 hours. The organic sheath is visible in figs. 27, 28, and 29.

Beneath the organic sheath is a single layer of flat-lying calcite rhombohedrons. This outermost pavement-like layer of calcite is not directly visible in the micrographs, but indications of it can be seen beneath the surface of the thin organic sheath in fig. 28. Lynts and Pfister (1967), using carbon replica techniques on the tests of various foraminifers from the Dry Tortugas, showed a single layer of calcite rhombohedrons overlying an inner layer of randomly oriented crystallites in A. angulatus. Fractured specimens observed in the scanning electron microscope indicate that except for the outer pavement-like layer, the remainder of the chamber wall is composed of randomly oriented calcite crystallites (see figs. 29 and 30). In fig. 29, the organic sheath as well as the underlying single layer of calcite rhombohedrons can be seen to extend over the sides and bottoms of the pits as well as over the flat portions of the test exterior. Fig. 30 shows the underlying randomly oriented layer exposed by ultrasonic treatment. Difficulty in focusing on fractured surfaces of the chamber walls suggested the presence of organic material within the wall as well as calcite.

Several specimens were etched in acidic sea water to reveal the internal morphology of the chamber walls. The surface of an etched specimen is seen in fig. 32 and at higher magnification in fig. 31. Removal of the calcite by partial dissolution reveals an intricate network of fibrous

material thought to be organic in composition. The darker areas in fig. 31 are shallow depressions representing the loci of pits on the original surface. In reflected light the partially dissolved tests appear extremely smooth and opaque, and are white in color.

Complete dissolution of the test leaves behind the cytoplasm encased in an organic liner (see fig. 2). The organic liner reveals numerous depressions which compliment the pits visible on the test surface (see fig. 33). At higher magnification (fig. 34) the chamber lining reveals a surface characterized by a network of organic fibers. It is not known if the chamber lining is a separate layer (it could not be seen as a separate entity in fractured tests) or if it represents an enrichment of organic material at the base of the chamber wall.

In summary, the scanning electron microscope reveals that the test of A. angulatus has a complex ultrastructure. The exterior surface of the test is covered with a thin organic sheath which exhibits no surface structure and is probably secreted by the pseudopods. Directly beneath the organic sheath lies a single pavement-like layer of calcite rhombohedrons arranged in flat-lying groups of sub-parallel crystallites. Below the pavement-like layer is the massive portion of the chamber walls, composed of a mixture of intertwining organic fibers and randomly oriented needle-like calcite crystallites. The basal portion of the chamber walls is entirely organic and is probably in contact with the cytoplasm (assuming that a thin layer of calcite does not line the chamber interior). The basal organic membrane appears to have a more regular pattern of fibers than does the organic matrix.

Angell (1967a) observed that in Rosalina floridana, two distinct types of organic membrane occur as part of the chamber wall, each with its peculiar staining properties. A thin "structureless" organic membrane occurs within the chamber wall separating layers of calcite. These thin membranes are extensions of the organic pore processes and are thought to strengthen the test by binding together several layers of calcite crystals. Most descriptions of the wall structure of Rosalina floridana do not mention the presence of the thin membranes. The basal membrane of each chamber wall, however, is relatively thick and is composed of "beaded" organic fibers. In a general sense, the thin membranes within the calcite wall of R. floridana correspond to the organic matrix in the wall of A. angulatus, and the basal membrane or chamber lining in R. floridana corresponds to the basal membrane in A. angulatus.

C. Decalcification.

Several specimens were decalcified with the purpose of inducing chamber formation. A. angulatus is able to survive total immersion in sea water lowered to pH 2.0 with concentrated hydrochloric acid for periods of one week and longer. The test is completely decalcified within 2 to 3 days. The last formed chamber decalcifies in a few hours, followed by the entire surface of the test exterior. The last portion to decalcify is the earliest formed chambers which form the inner core of the test. While in acidic sea water, the pseudopods are fully retracted and the foraminifer appears inactive. The last one or two chamber rows are empty but the organic membrane is visible.

Plate 10

Figure 12 Normal cytoplasm of a specimen not engaged in chamber formation. MT = mitochondrion. V = vacuole.
MB = basal organic membrane lining chamber interior.
Z = zoochlorellae. X = membraneous structure.

Transmission electron micrograph. X 9,900.

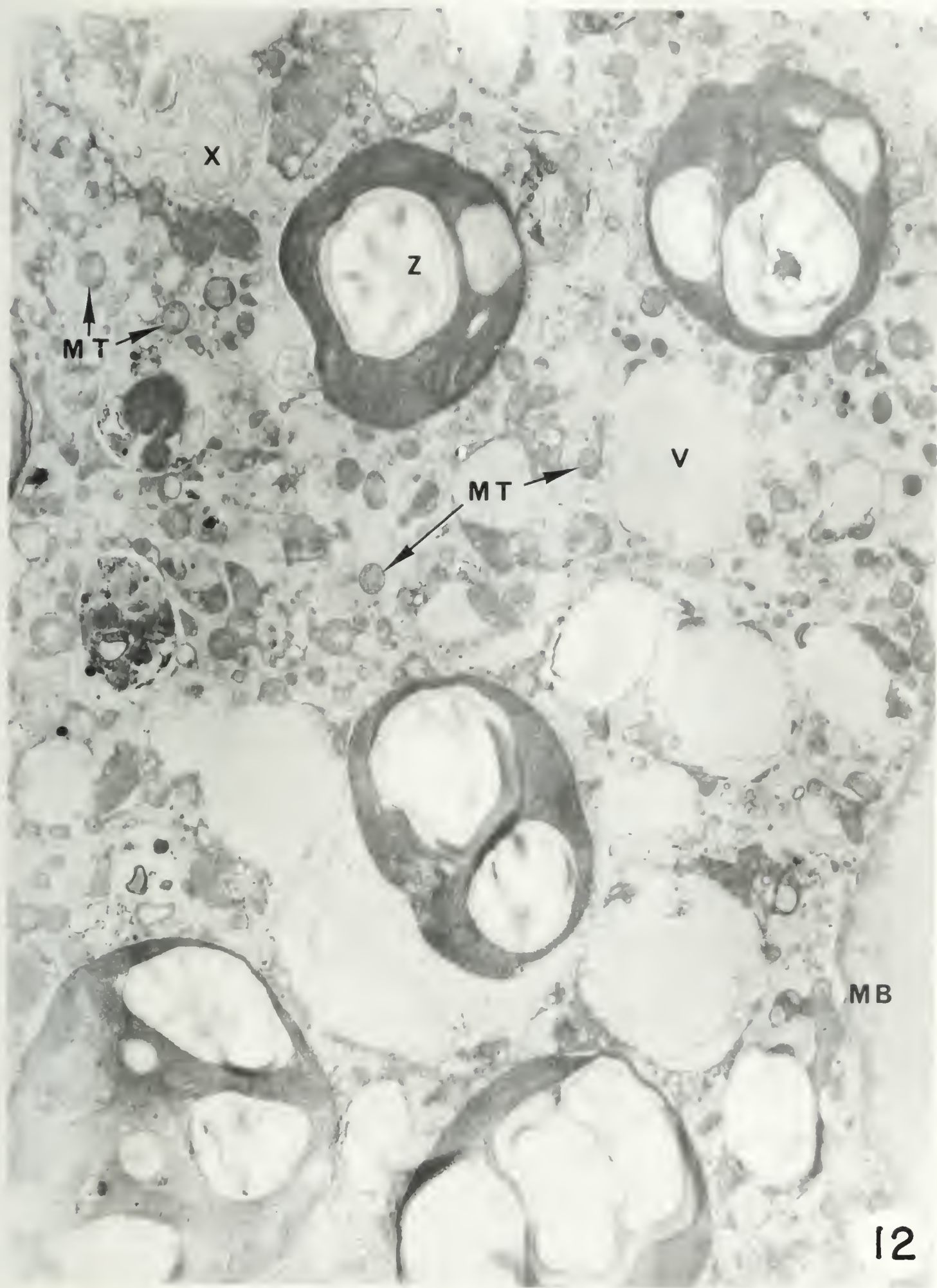


Plate 11

Figure 13 Normal cytoplasm. Complex, dense cytoplasm is characteristic of foraminifers. MT = mitochondrion. V = vacuole. Z = zoochlorellae. MB = basal organic membrane lining chamber interior. Note fuzzy appearance after decalcification. Plasmalemma of cytoplasm lies adjacent to the basal organic membrane.

Transmission electron micrograph. X 16,200.

14 Zoochlorellae may occupy most of the chamber volume and preferentially occur adjacent to the outer chamber walls. MB = basal organic membrane.

Transmission electron micrograph. X 9,000.

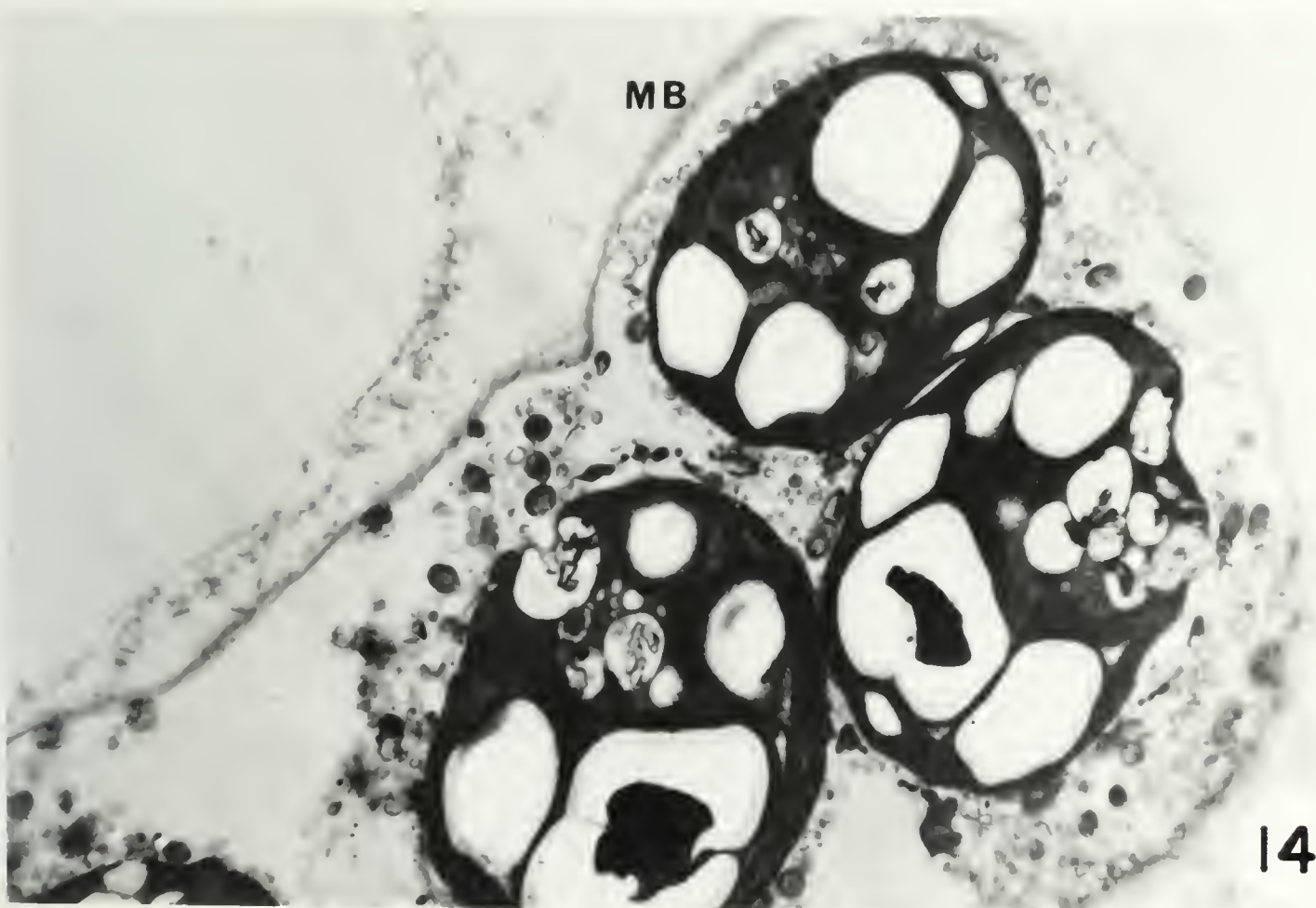


Plate 12

Figure 15 View from transparent cyst wall (X) to outer portion of organic membrane (L). Space between cyst and forming chamber may contain apparently free floating vesicles, seen in a linear arrangement in this micrograph. A few mitochondria are also present.

Transmission electron micrograph. X 10,500.

16. Detail of transparent cyst wall. Cyst is composed of secreted fibrillar material and a few vesicles and mitochondria. F = Fibrous groundplasm.

Transmission electron micrograph. X 48,100.

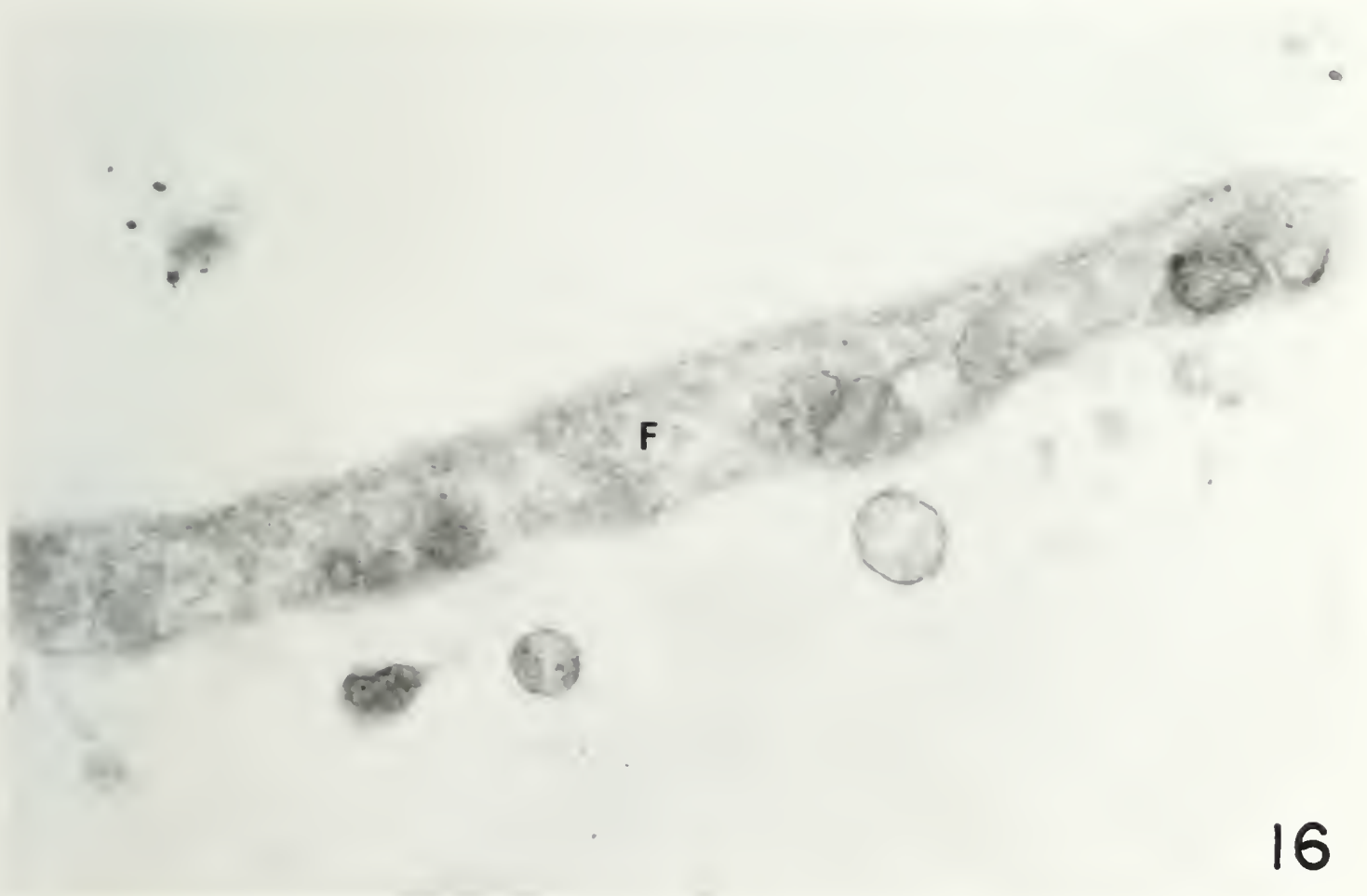
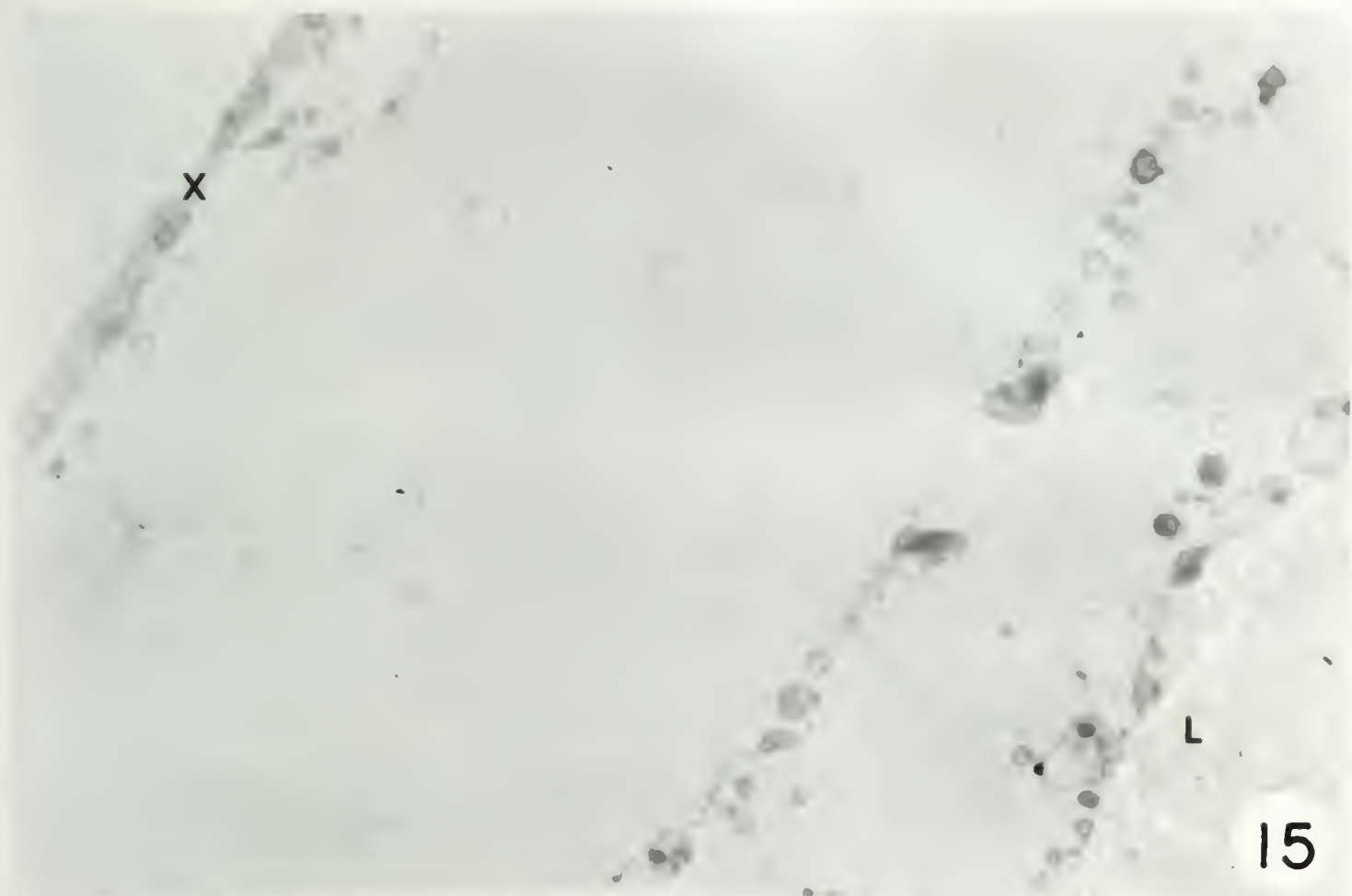


Plate 13

Figure 17 Anlage cytoplasm is composed mostly of electron light vesicles with occasional membrane bound strands of cytoplasm consisting of a fibrillar groundmass and mitochondria. MT = mitochondrion.

Transmission electron micrograph. X 37,200.

18 Pseudopod interior while organic membrane is forming
G = oval granule thought to release the fibrous material
(F). MT = mitochondrion.

Transmission electron micrograph. X 19,750.

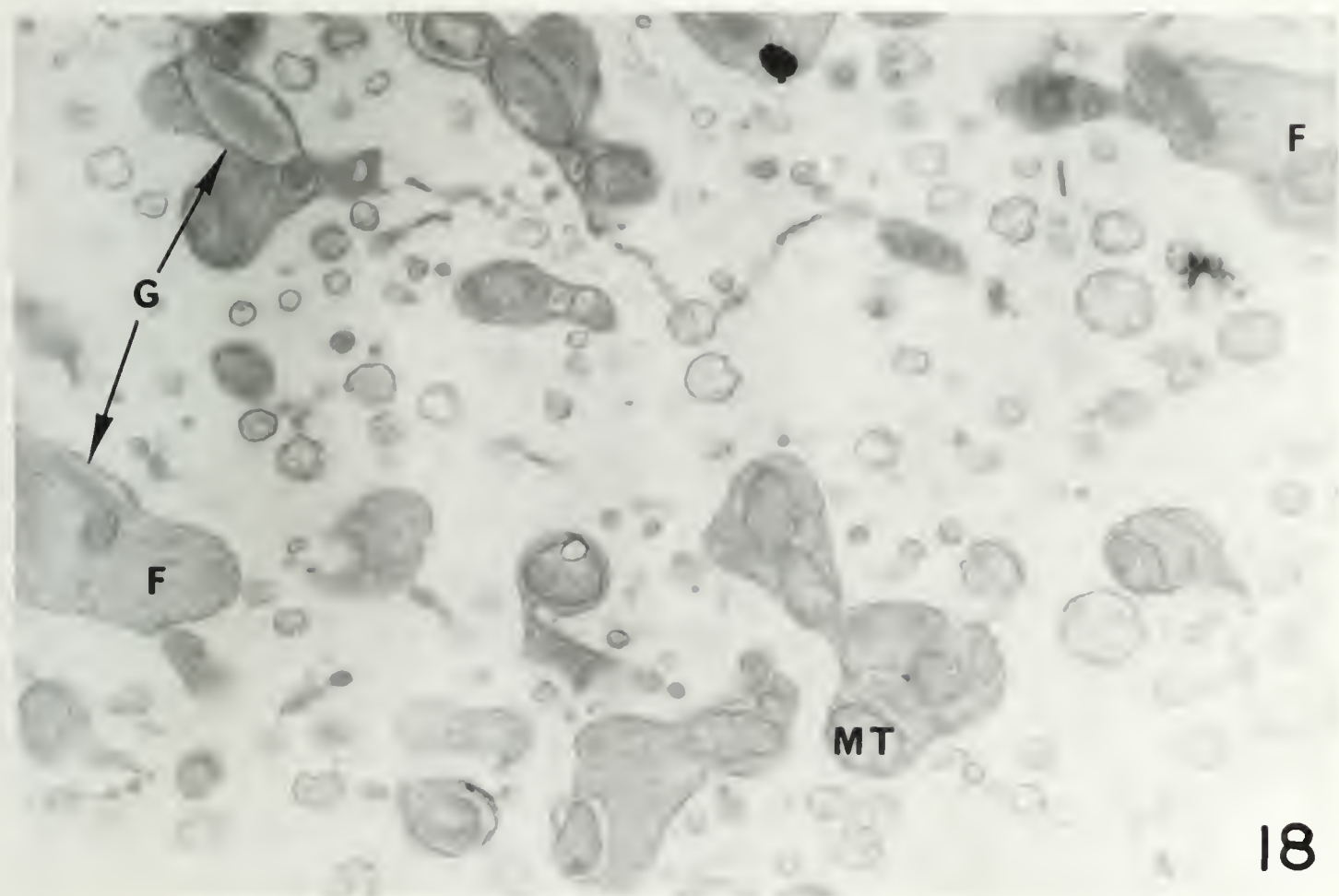
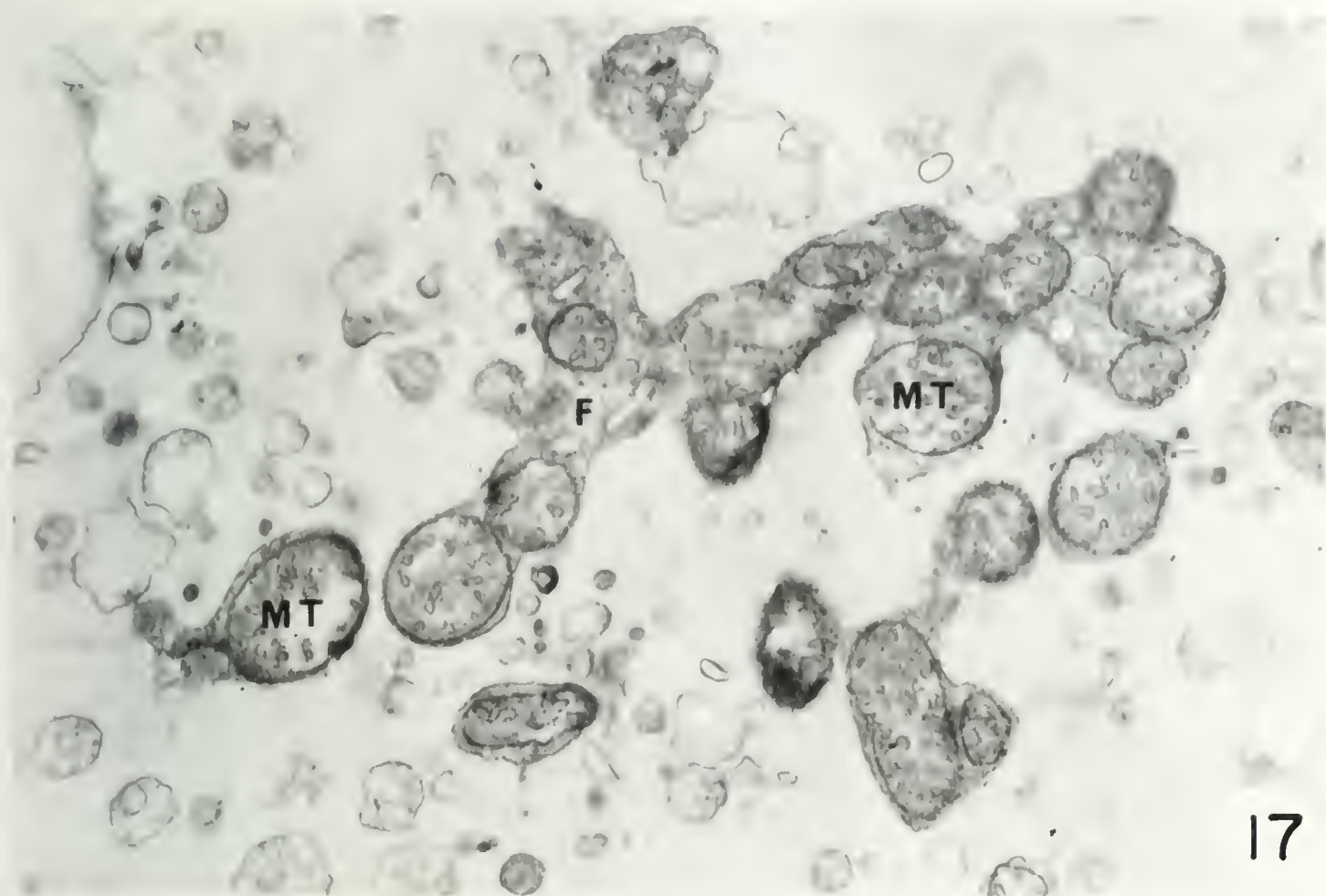


Plate 14

Figure 19 Chamber interior while organic membrane is forming. VS = vesicles containing a few fibers are thought to be empty, having given up their fibrillar material to the organic membrane. Mitochondria are still present in the vesicles. MB = organic lining of chamber interior.

Transmission electron micrograph. X 42,250.

20 As calcification proceeds, dense needle-like structures thought to be calcite appear in the organic matrix (L). Left side of micrograph is a portion of a pseudopod on the outside of the organic membrane. Arrow indicates oval granule emptying its fibrillar contents (F) into a vesicle.

Transmission electron micrograph. X 19,350.

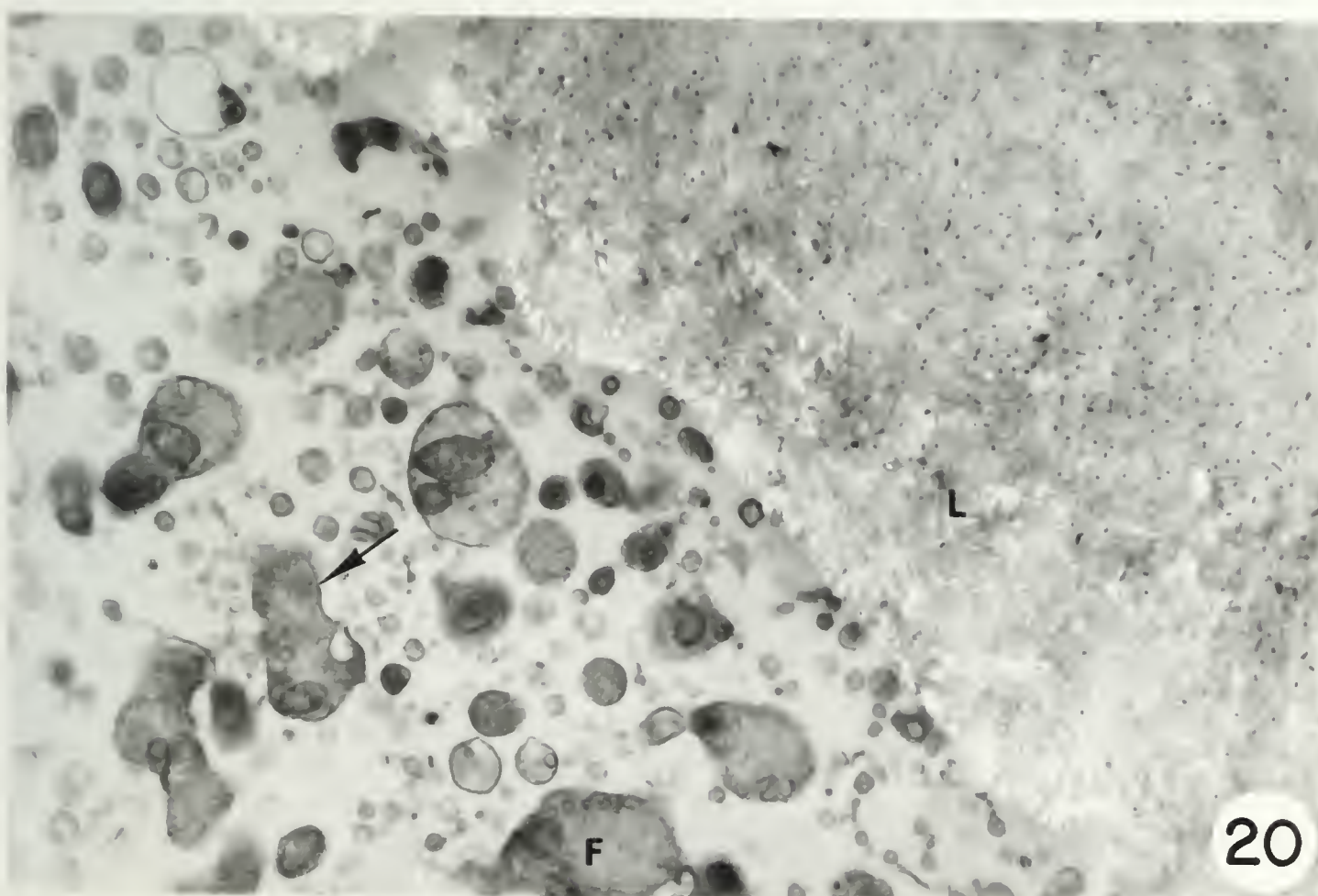
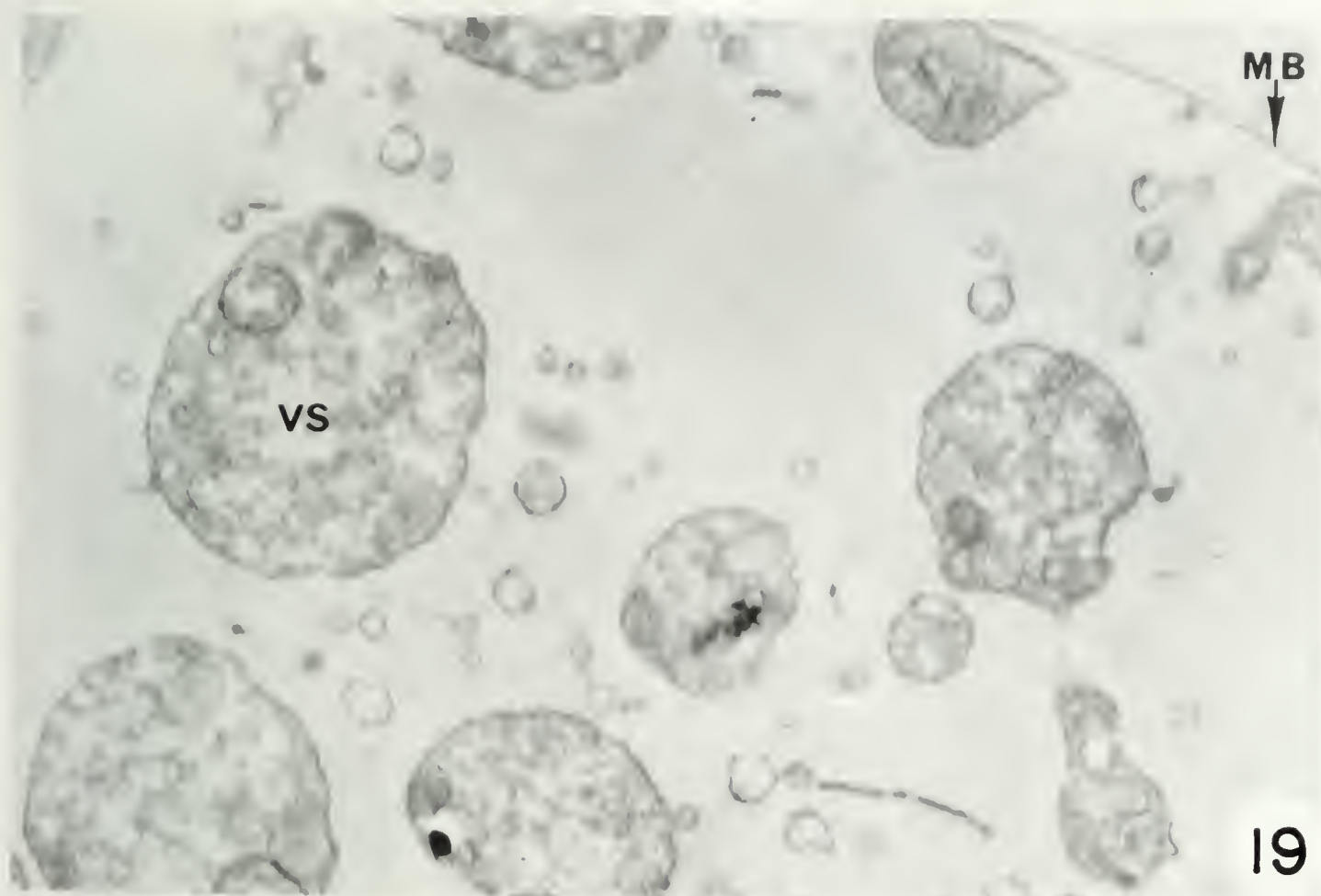


Plate 15

Figure 21 Invading cytoplasm is less dense than normal but has numerous zoochlorellae (Z). F = fibrous material is still present in the chamber. MT = mitochondrion. L = organic membrane.

Transmission electron micrograph. X 19,300.

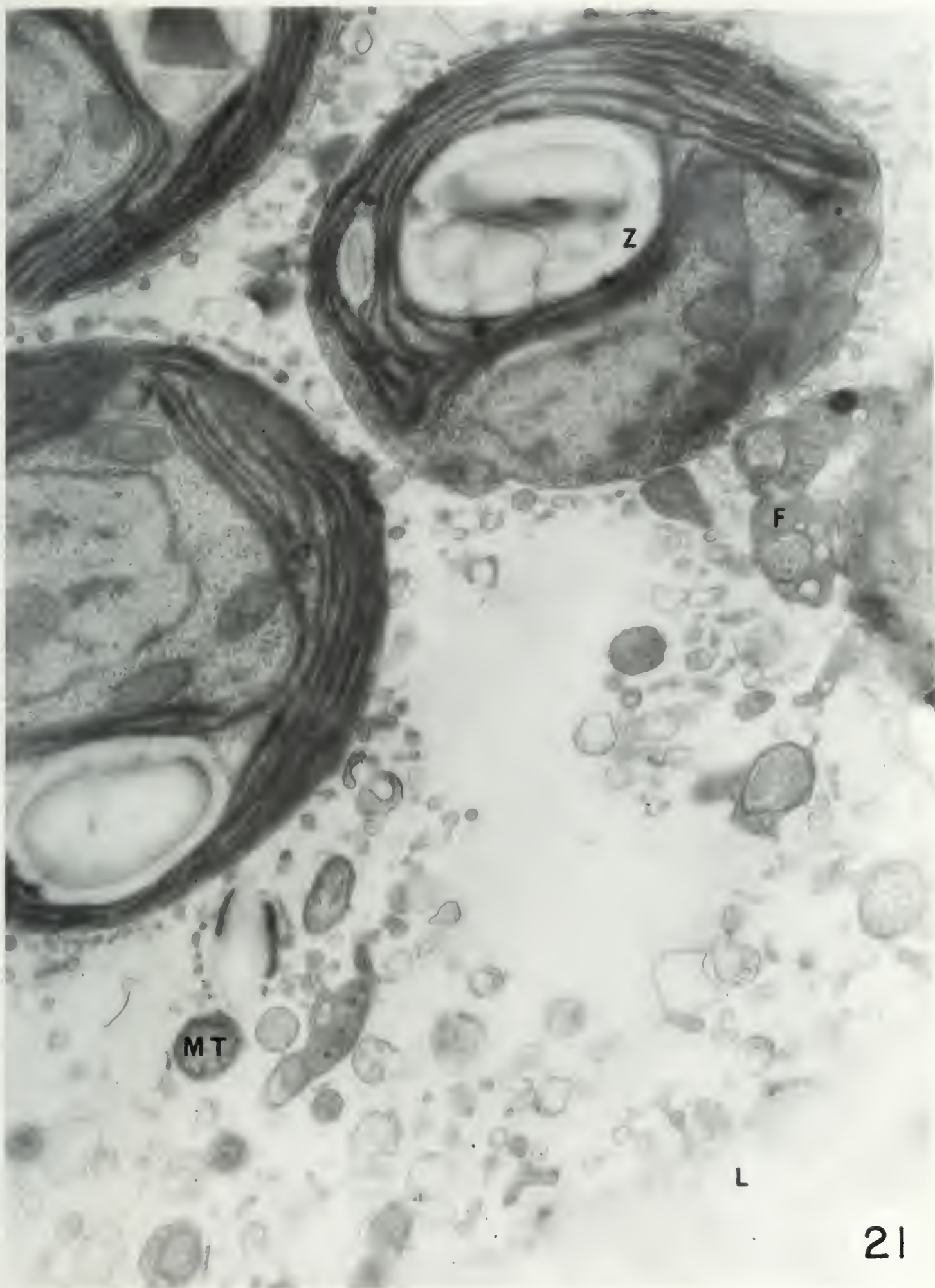


Plate 16

Figure 22 MT = mitochondria in invading cytoplasm. Mitochondria are still in association with fibrillar material. Zoochlorellae (Z) are more numerous adjacent to the organic membrane than in the chamber interior.

Transmission electron micrograph. X 15,400.

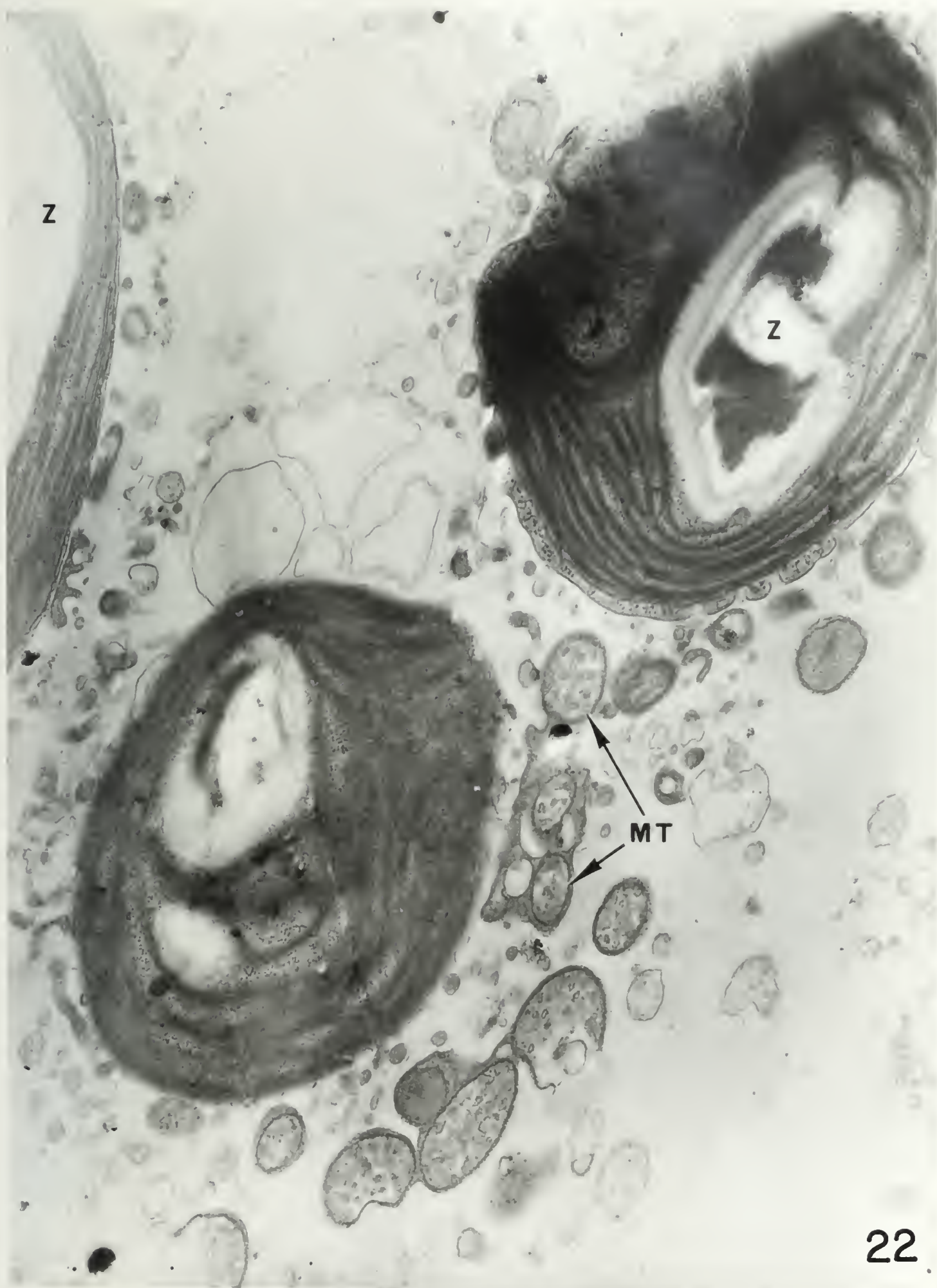


Plate 17

Figure 23 *Zoochlorella* viewed at high magnification.
PY = pyrenoid. MT = mitochondrion. C =
chloroplast with grana. N = nucleus.
Arrow indicates pore in double nuclear
membrane.

Transmission electron micrograph. X 28,400.



Plate 18

Figure 24 Detail of test surface in umbilical region. Pits are superficial features, not pores.

Scanning electron micrograph. X 550.

25 Apertural face with multiple apertures.
Each aperture is surrounded by a raised lip.

Scanning electron micrograph. X 750.

26 Fractured test. Surface of test at top of micrograph.
Cytoplasm in chamber interior is visible. Arrows
indicate zoochlorellae. Fixed and freeze-dried.

Scanning electron micrograph. X 600.

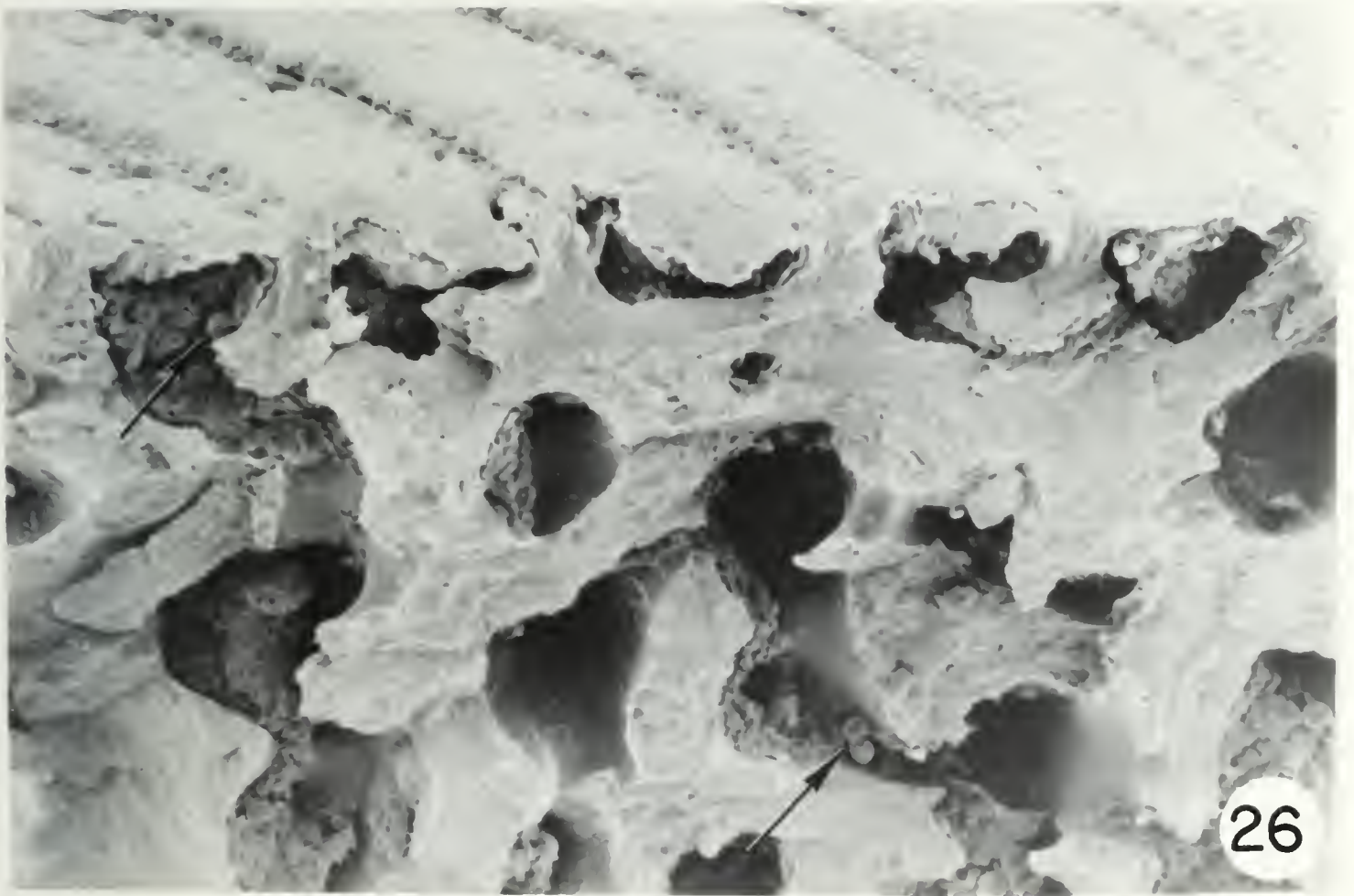
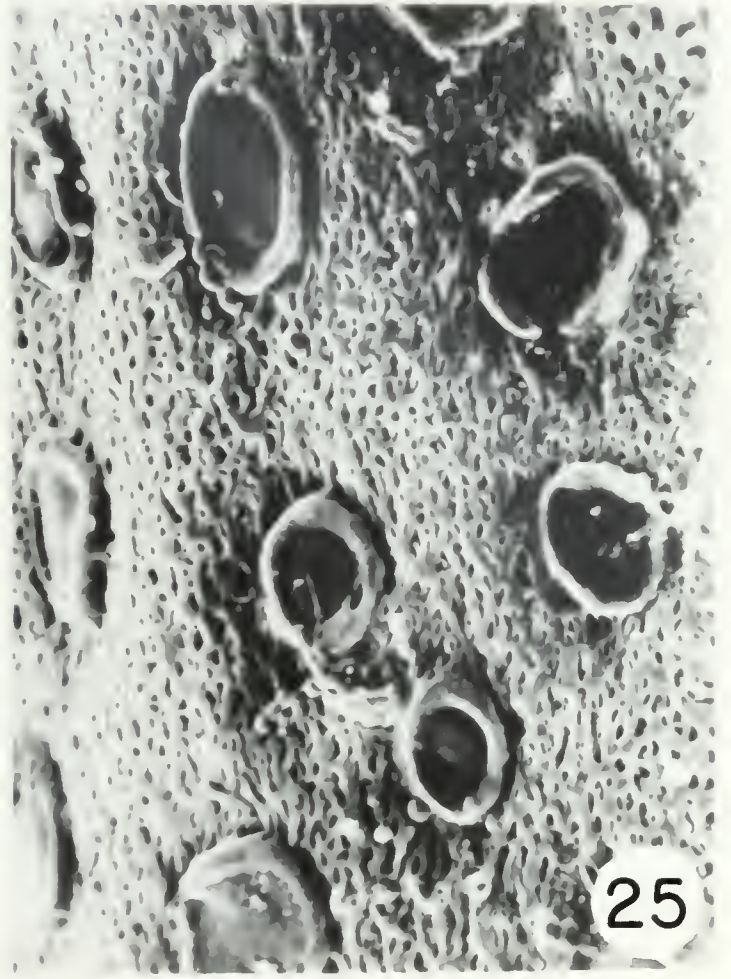
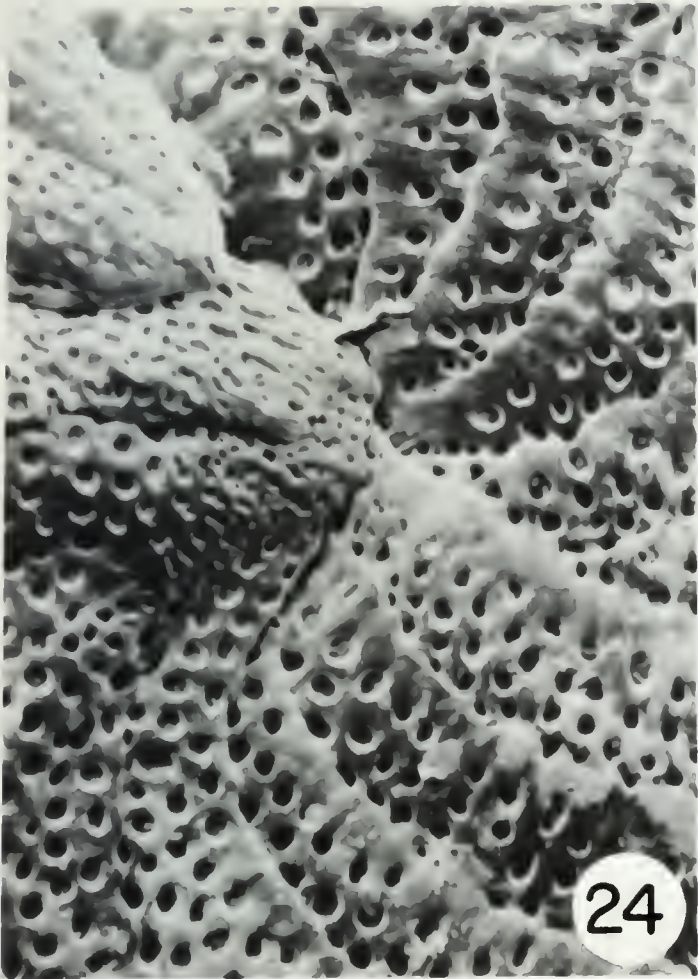


Plate 19

Figure 27 A thin organic sheath covers the test surface. Chemical attack of the underlying calcite is greatly retarded when sheath is present. Fixed and freeze-dried.

Scanning electron micrograph. X 2100.

28 Detail of fig. 27. Indications of the underlying pavement-like layer of calcite rhombohedrons are visible through the thin membrane. Fixed and freeze-dried.

Scanning electron micrograph. X 20,500.

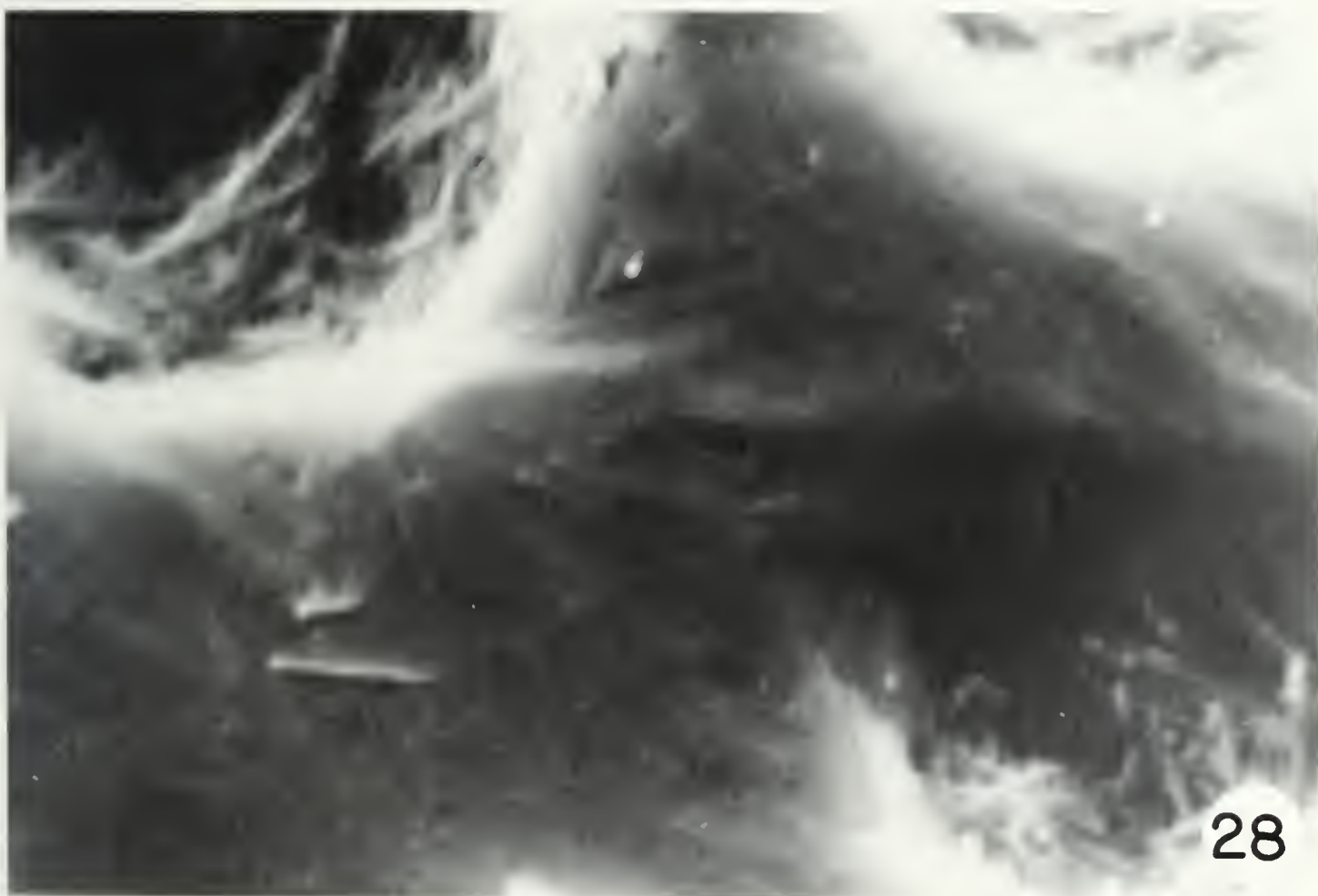
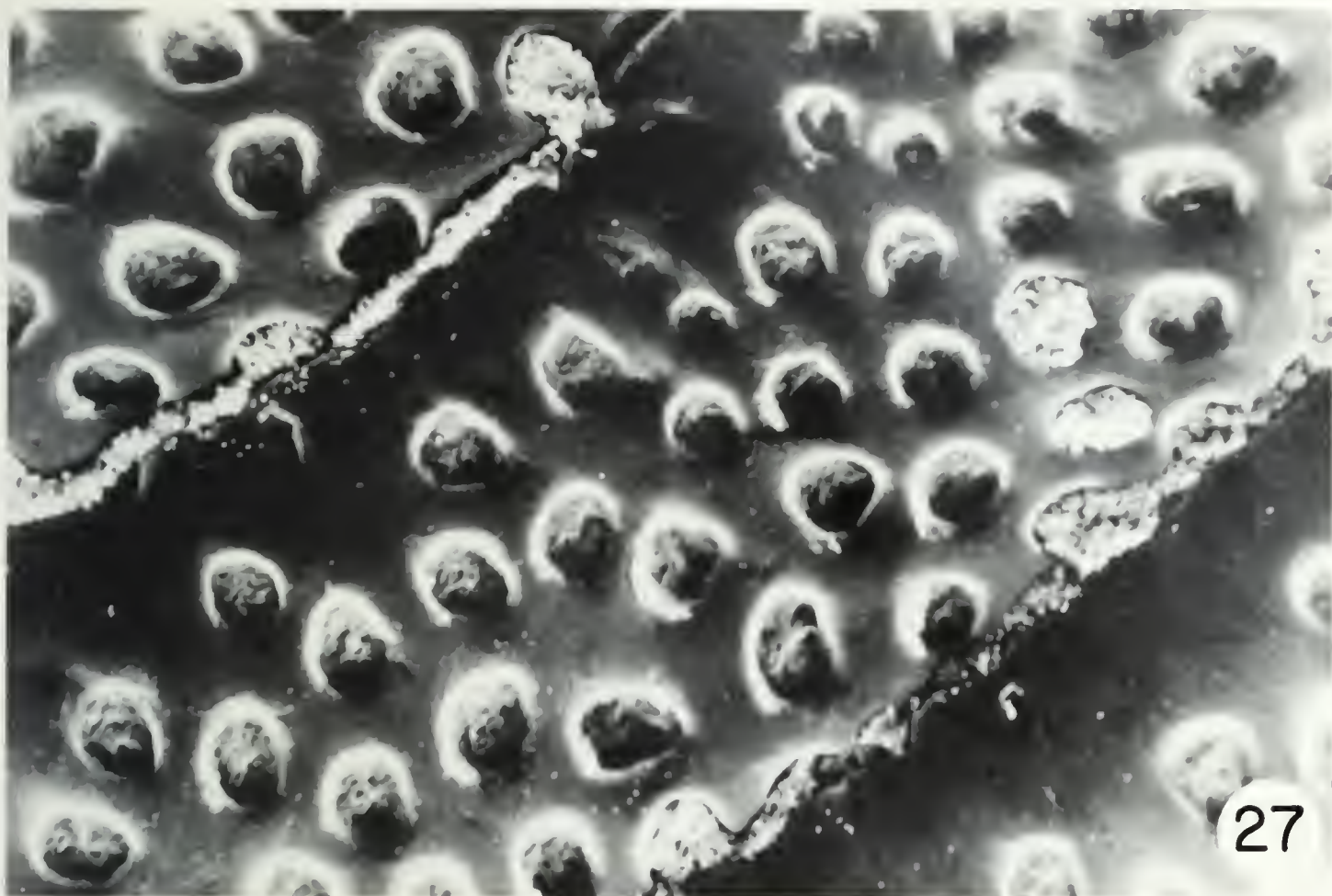


Plate 20

Figure 29 Fractured edge of the test. Thin organic sheath covers test surface including sides and bottoms of pits. Massive test wall is composed of randomly oriented calcite crystals. Fixed and freeze dried.

Scanning electron micrograph. X 11,300.

30 Same view as in fig. 29. Specimen cleaned with ultrasonic treatment to remove surface layers. Randomly oriented layer of calcite exposed on test surface and in fracture wall.

Scanning electron micrograph. X 11,300.

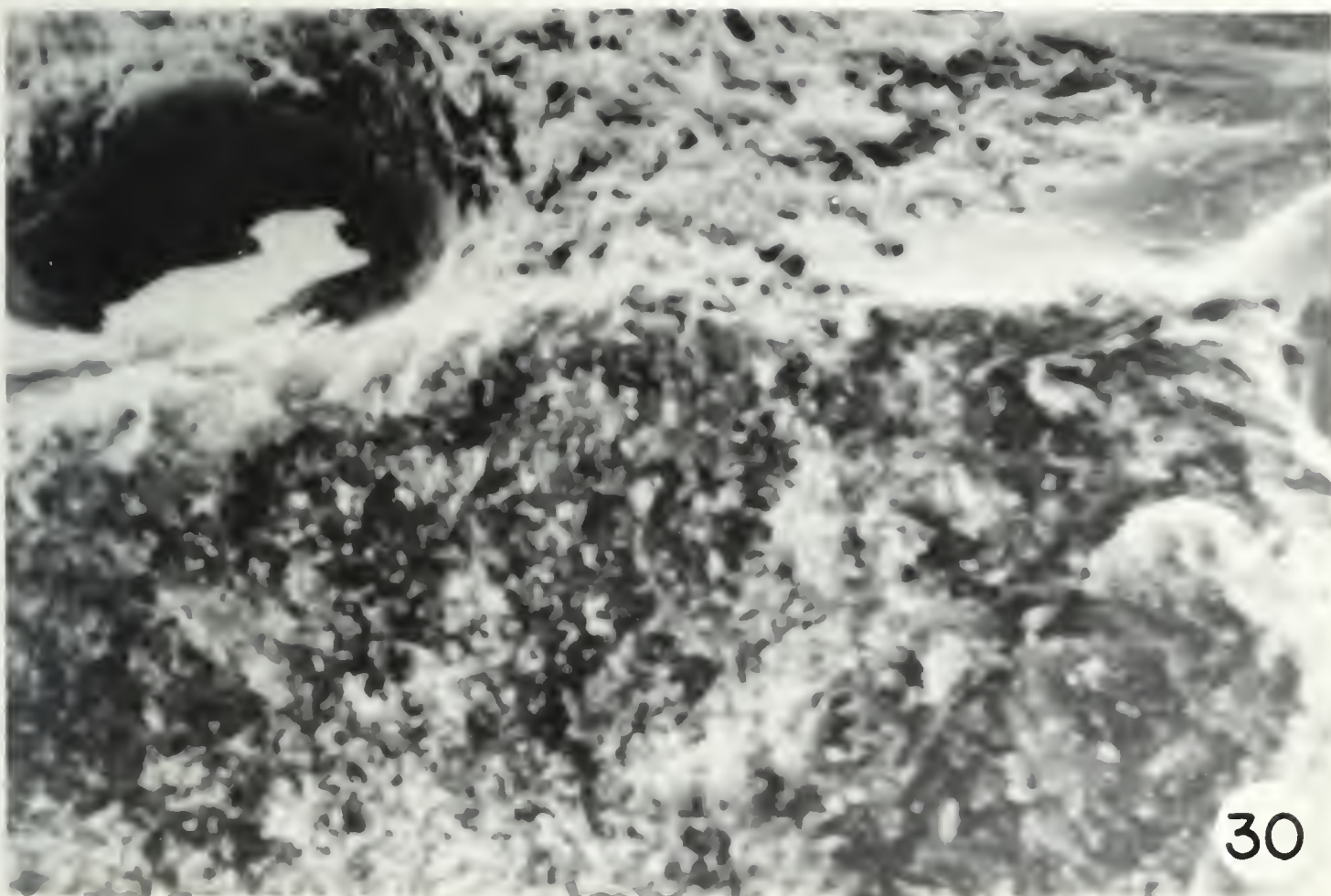
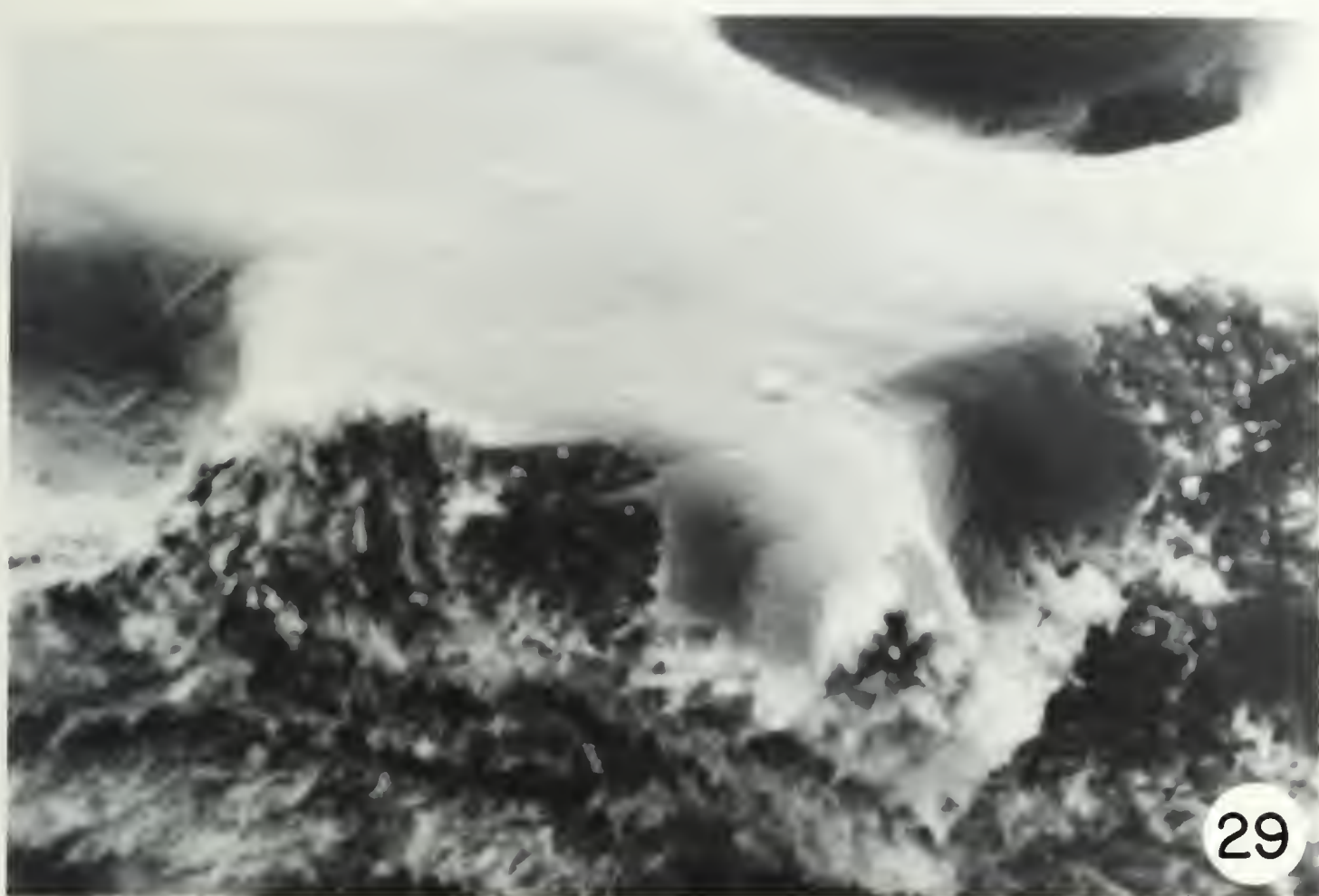


Plate 21

Figure 31 Detail of test surface after partial decalcification in sea water of pH 2.0. A mesh of organic fibers remain after calcite dissolves. Dark areas indicate loci of pits. Fixed and freeze dried.

Scanning electron micrograph. X 10,400.

32 Etched test surface. Organic fibers are visible at surface. Depressions reflect loci of pits on original surface.

Scanning electron micrograph. X 1100.

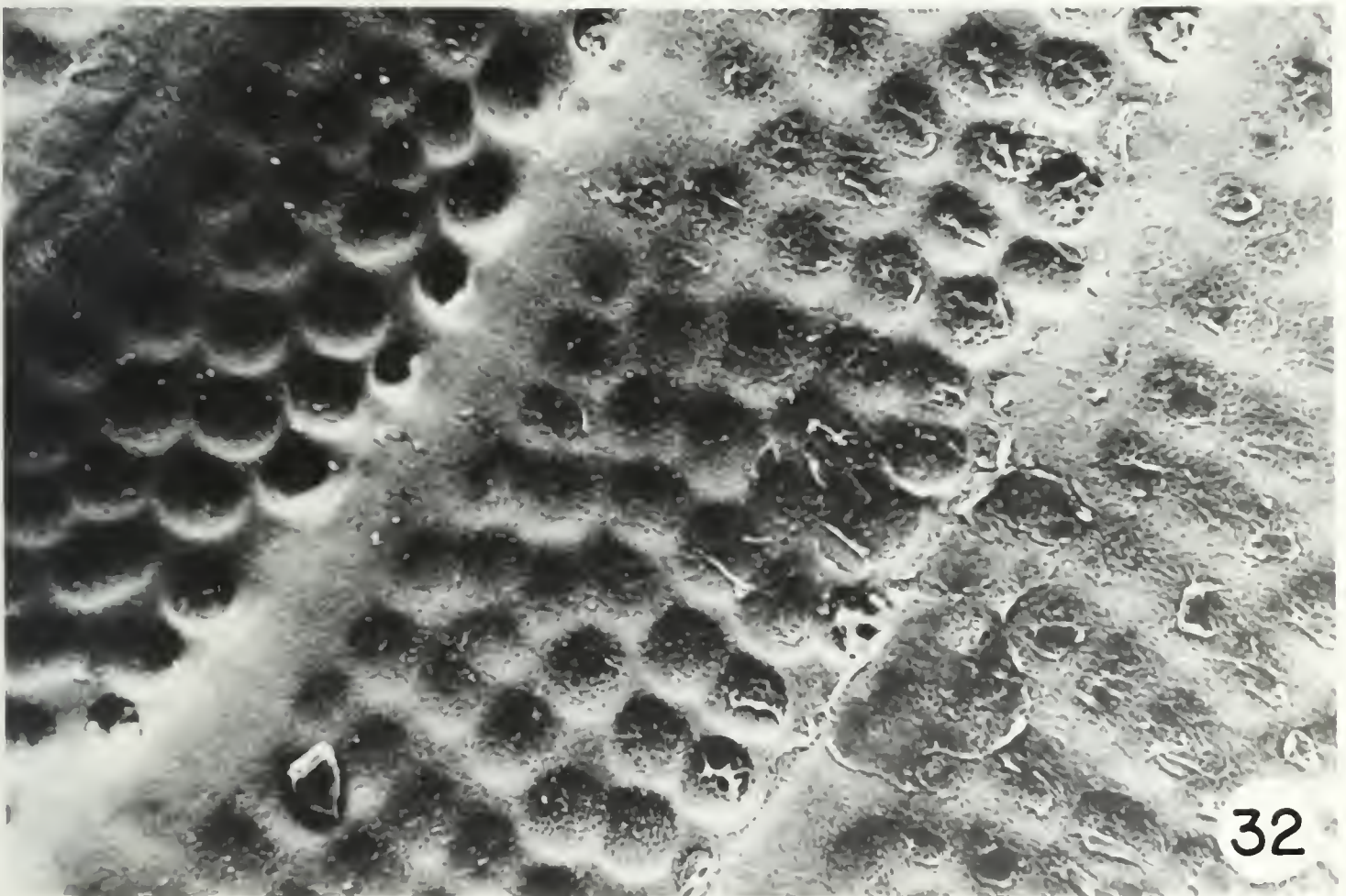
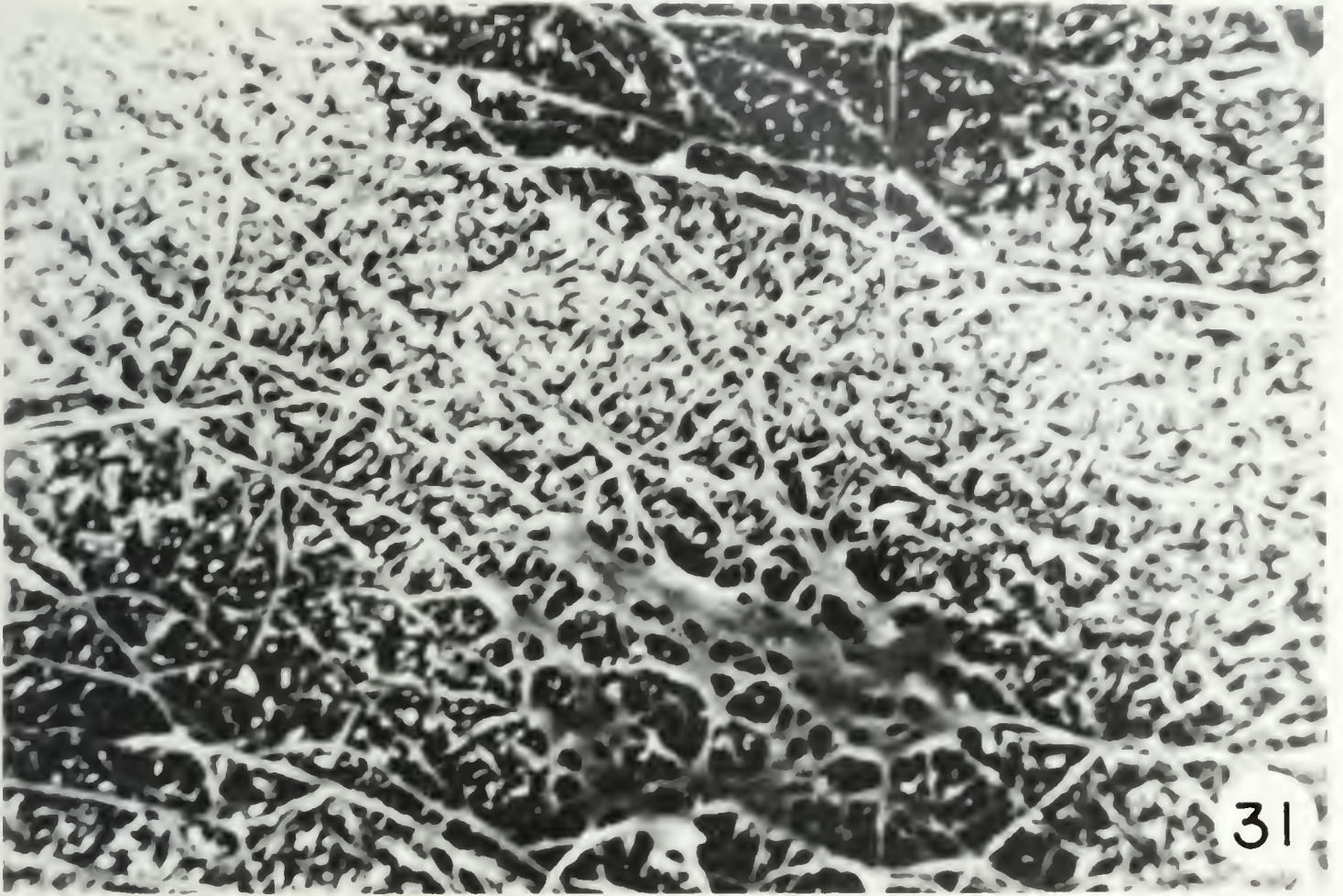


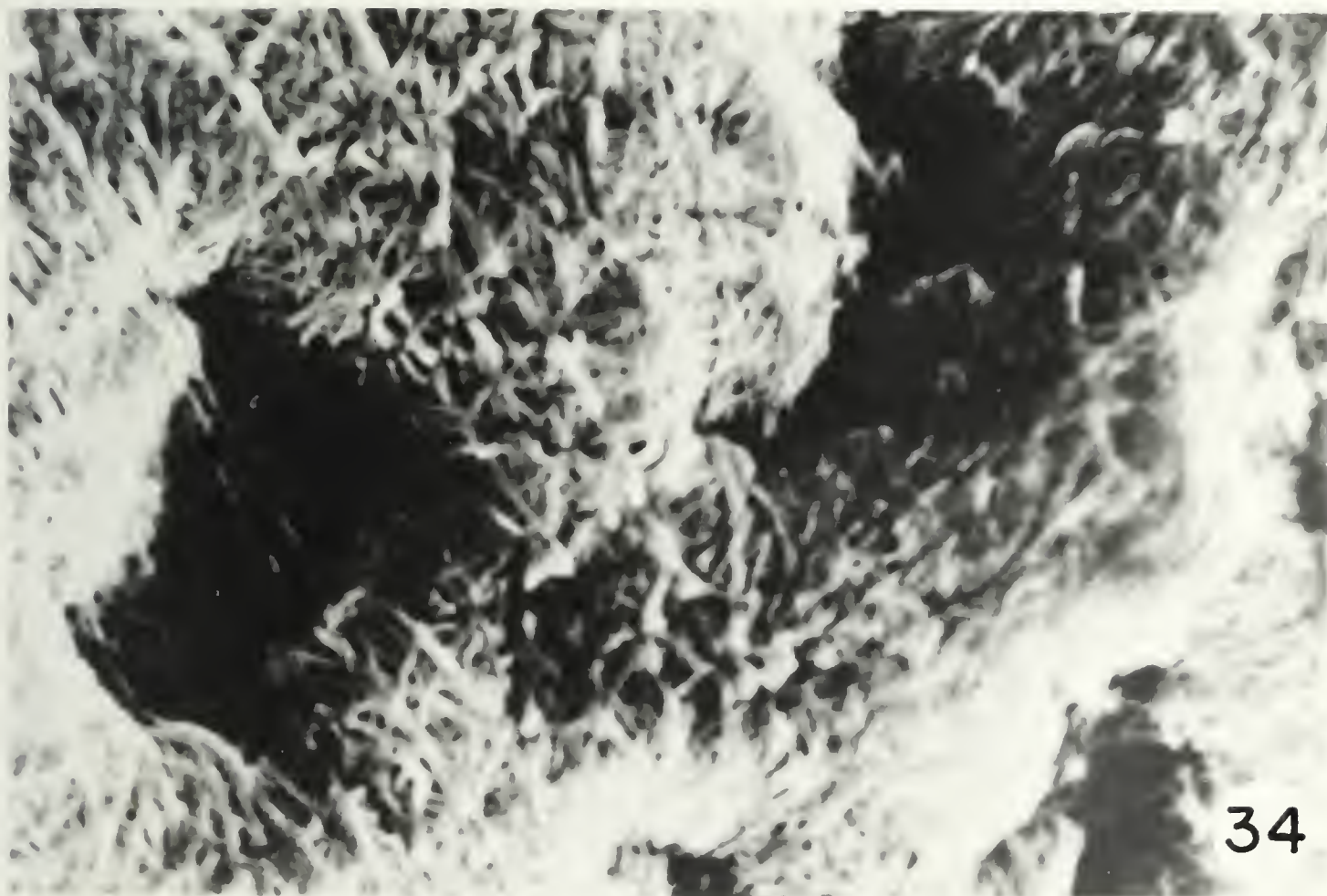
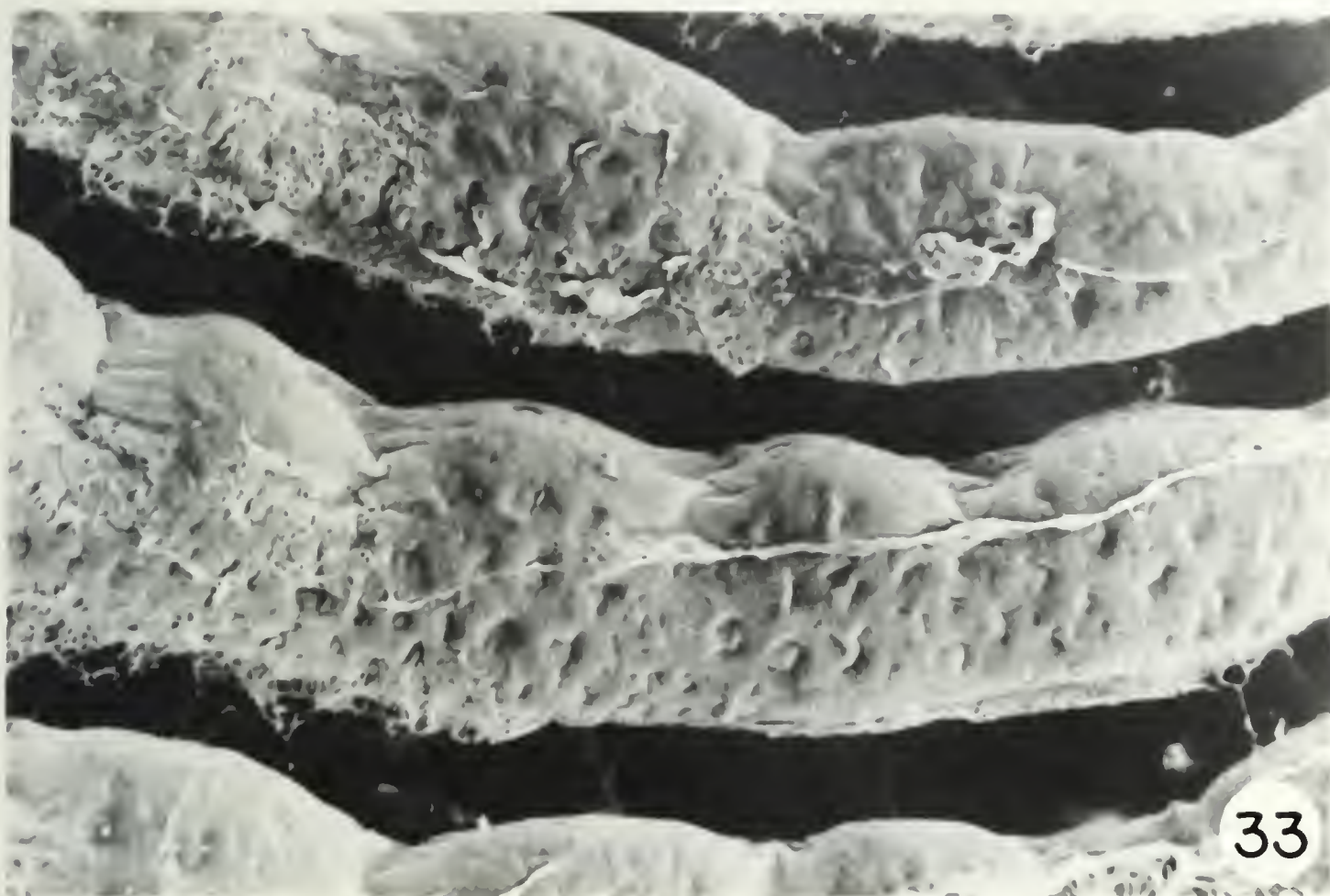
Plate 22

Figure 33 Complete decalcification leaves an organic lining within each chamber. Depressions on surface indicate that protuberances on chamber interior compliment pits on test surface. Fixed and freeze-dried.

Scanning electron micrograph. X 1100.

34 Detail of fig. 33. Organic fibers form a pattern on surface of chamber lining.

Scanning electron micrograph. X 11,000.



After transfer to normal Florida sea water of pH 8.2, the organism recovers rapidly and sends out pseudopods. All decalcified specimens recalcified their tests within one week after transfer to normal sea water. The recalcification, however, was incomplete resulting in abnormal tests. Green cytoplasm was observed outside of the test of several decalcified specimens. In all other observations of normal specimens, the zoochlorellae remained at all times within the tests of the foraminifers. In decalcified specimens, cytoplasm with zoochlorellae occurred as an amoeba-like mass adjacent to or on the test, or within the pseudopods. This condition is considered abnormal and indicates that the organic membrane had ruptured, or that the calcified test aids the organism in modifying the cytoplasm.

All specimens which had recalcified their tests remained viable; none, however, were observed to form new chambers.

DISCUSSION

A. Comparison to previous work.

The process of chamber formation in A. angulatus differs significantly from that observed by Angell (1967b) in Rosalina floridana. The differences were expected since the two foraminifers belong to separate taxa characterized by differences in wall structure of the test. It was partially on the known differences in wall structure that A. angulatus was selected as a subject for this study.

Foraminifers which construct calcareous tests are subdivided into two major groups: the "imperforate" and the "perforate", depending on the presence or absence of pores which extend through the test wall. Further classification is based on the microstructure of the walls. The imperforate foraminifera are commonly referred to as "porcelaneous", a term first applied by Williamson in 1858. The term porcelaneous describes the test as it appears in reflected light, white and opaque. Another diagnostic feature of porcelaneous tests is the amber color seen when viewed in transmitted light. The amber color has puzzled investigators for years as it is independent of the microstructure of porcelaneous tests, and does not occur in perforate calcareous tests. This study indicates that in Archias, a porcelaneous foraminifer, the amber color observed in transmitted light is due to the mixture of organic fibers and calcite within the test walls.

Porcelaneous tests are also characterized by the lack of an interference figure in polarized light, commonly observed in perforate

calcium carbonate tests. The lack of an interference figure can be explained by the mutual compensation effect produced by the random orientation of many calcite crystals.

Rosalina floridana is a perforate foraminifer. In general, it can be said that the mechanics of chamber formation differ significantly in Archias and Rosalina; the ultrastructure of the cytoplasm associated with comparable events during chamber formation, however, shows a high degree of similarity.

The mechanics of chamber formation in A. angulatus and R. floridana differ significantly in several aspects. Rosalina deposits a new layer of calcite over the entire test surface as each new chamber is added; in Archias each new chamber is added as a separate entity without calcifying the remainder of the test. This difference results in a relatively massive wall structure in Archias, and a distinctly layered wall structure in Rosalina, the number of layers increasing with age of the chambers.

Rosalina does not form the pseudopodial array illustrated in fig. 5 and characteristic of chamber formation in Archias. The absence of the pseudopodial array is probably related to chamber form. In Rosalina about 15 chambers coiled in a low spire are present in the adult, the last chamber formed having a volume about equal to that of the remainder of the test, and the chamber is formed on the substrate. In Archias the chamber row is formed over the apertural face, and because of the shape of the test, the forming chamber does not rest on the substrate. The pseudopods may support the chamber as it is forming against the more or less vertical apertural face.

In Rosalina, the chamber wall increases in thickness as the calcite layer is deposited over the thin basal organic membrane. Calcification in Archias appears to occur within a relatively thick organic membrane rather than over it. The result is that the chamber wall does not increase in thickness as calcification proceeds. Arnold (1964) noted that during chamber formation in Spiroloculina hyalina, a miliolid porcelaneous foraminifer, the thickness of the secreted chamber wall remained constant throughout the calcification process. The thickness of the secreted organic membrane was equal to the thickness of the calcified wall. He concluded that the organic membrane was a true organic matrix rather than a basal layer of the chamber wall. Light microscopic observations on Articulina mucronata, another miliolid foraminifer, during this study led to the same conclusions; calcification occurs within the organic membrane without increase in chamber wall thickness. Similar conclusions were reached by Lynts and Pfister (1967) while observing calcification in Articulina sagra.

The time required for chamber formation also differs in Archias and Rosalina. Chamber formation is completed in less than 24 hours in Rosalina (Angell, 1967b); Archias requires 2 to 4 days for complete calcification to occur. The longer time seems to be characteristic for foraminifers building porcelaneous tests (Angell, personal communication). Articulina mucronata is occasionally collected in south Florida with the last chamber in a non-calcified state. Several such specimens when placed in culture completed calcification in 2 to 3 days, without formation of a growth cyst.

The ultrastructure of the cytoplasm associated with several comparable stages of chamber formation, is similar in R. floridana and A. angulatus. In both foraminifers, extensive modification of the normal cytoplasm occurs during chamber formation.

Angell (1967b) found that the anlage cytoplasm is mostly composed of structureless vesicles with occasional mitochondria. He interpreted the highly vesiculate anlage cytoplasm as a passive structure, which only served as a substrate upon which the organic membrane was secreted. In Rosalina a relatively large volume of cytoplasm is needed for the anlage as the new chamber is frequently equal in volume to the earlier test. In Archias, the anlage cytoplasm is highly vesiculated and also served as a substrate for secretion of the membrane by the pseudopods and associated dense strands of cytoplasm. Formation of highly vesiculate cytoplasm is common in foraminifers. The final chamber of many benthonic foraminifers is usually filled with veisculate cytoplasm; the dense normal cytoplasm occupies the inner chambers (Angell, 1967b; Marszalek, Wright, and Hay, 1967a, 1967b).

The structural material employed during secretion of the organic membrane by both foraminifers is a fibrous substance which is thought to be produced in the cytoplasm of the inner chambers and transported to the site of membrane formation by the pseudopods. The fibrous material is compressed or in some other way altered at the site of deposition since the membrane does not appear fibrous in thin section.

Oval granules occur in the modified cytoplasm of Rosalina as well as Archias. The oval granules are thought to represent prepackaged fibrous material in a compact, easily transportable form. Transitional stages from oval granule to fibrous cytoplasm have been observed by Angell in the cytoplasm of R. floridana, and in this study in the cytoplasm of A. angulatus.

The membranous chambers of both organisms are invaded by dense cytoplasm prior to calcification. In Archias the invading cytoplasm is characterized by numerous zoochlorellae. Algae were not present in the invading cytoplasm of specimens of Rosalina examined by Angell. Previous investigations by the author indicate that symbiotic algae are present in the cytoplasm of R. floridana, though not in large numbers as in Archias. Furthermore, the specimens used by Angell were descendents of specimens kept in laboratory culture for several years, and may have lost their symbiotic algae. Apparently the symbiotic algae are not necessary for calcification, but may facilitate its occurrence. It is sufficient to say that the invading cytoplasm in both foraminifers is distinct from that which occupies the chambers prior to calcification.

Another striking similarity in the modified cytoplasm of Rosalina and Archias is the association of mitochondria with the cytoplasm involved in chamber formation. Angell noted that during calcification in Rosalina, a sheath of vesiculate cytoplasm containing mitochondria as the only recognizable cell organelles covers the basal membrane upon which the calcite is deposited. In Archias, mitochondria are associated with

the vesiculate anlage, the fibrous material during secretion of the membrane, and with the invading cytoplasm during calcification.

B. The role of symbiotic algae in chamber formation.

The presence of zooxanthellae and zoochlorellae in foraminifers has been long suspected (Hedley, 1964), but only recently has been proven by electron microscopic examination of thin sections. Figure 23 shows the detailed structure of a zoochlorella in the cytoplasm of A. angulatus. There can be little doubt that the characteristic vivid green color of the foraminifer is due to the presence of thousands of unicellar algal cells. The usual assumption of investigators suspecting the presence of symbiotic algae in foraminifers, is that the host organism derives oxygen and nutrients from the metabolic wastes of the algae.

The possible importance of zooxanthellae and zoochlorellae to calcification in foraminifera should not be overlooked. Studies on the symbiotic relationship of zooxanthellae and hermatypic corals, suggests that the presence of algae exerts an important influence on the rate of calcium carbonate deposition by these reef building organisms (Goreau and Goreau, 1959). Goreau, 1961, has shown that the rate of calcium carbonate deposition in hermatypic corals varies directly with the amount of light present. The amount of mineral deposition on cloudy days is about 50% that which occurs on sunny days. Also, calcium carbonate deposition in corals which have been induced to expell their zooxanthellae is greatly reduced (about 10% of normal), and what little mineral deposition

does occur, does not vary with the light intensity. It is interesting to note that, without exception, all known species of coral capable of rapid calcium carbonate deposition (able to build reefs) are characterized by the presence of zooxanthellae in their tissues. Clearly Goreau has shown that the presence of unicellular algal cells within the host tissues, influences the rate of calcium carbonate deposition.

In a related study (Goreau and Goreau, 1960) the transfer of materials from symbiotic zooxanthellae to two species of reef building corals was measured by autoradiographic methods, using introduced radiocarbon as the traceable isotope. Results showed very little transfer of substances from the algal cells to the coral tissues, although radiocarbon uptake by the algal cells varied directly with the light intensity. The coral polyps apparently do not digest the algae or their waste products. In contrast to closely related noncalcifying colenterates, corals receive little or no nutrient from their symbiotic zooxanthellae. The algae are thought to increase the metabolic efficiency of their host, possibly through minor secretions of "enzyme-like" substances. They concluded that the zooxanthellae exert an enormous influence on the calcium deposition rate in hermatypic corals, but that the algae do not contribute significantly to the nutrition of the coral.

A study by Lee and Zucker (1969) indicates that the algal symbiont in Archias angulatus facilitates calcium uptake by that foraminifer. Calcium isotope uptake was compared in specimens with the algal symbiont to specimens without the algae. The test specimens were fed bacteria and

several species of diatoms. Their results showed that calcium uptake was greatest in Archias with the symbiotic zoochlorellae and that more labeled calcium was incorporated in light than in the dark, although calcium uptake did not vary directly with increased photosynthetic activity of the algae. They also noted that in starved cultures of Archias with the algal symbiont, radiocarbon was retained by the symbionts indicating that the algae were not used as a food source.

The above mentioned experiments indicate that the presence of zoochlorellae in A. angulatus may facilitate calcification during chamber formation. The examination of thin sections in this report confirms the observations of Angell (1967), that a foraminifer is capable of modification of its cytoplasm during chamber formation. It therefore seems unlikely that the zoochlorellae would be found within the highly modified cytoplasm closely associated with calcification unless their presence was dictated by the foraminifer. If the observation by Goreau (1961) that algae enhance calcification in hermatypic corals by enzyme secretion is accurate, then perhaps there is an advantage to the foraminifera to have the enzyme producer at the active site of calcification -- the newly formed chambers in Archias.

Although not proven by direct observation, the results of Lee and Zucker (1969) suggest that in foraminifers without symbiotic algae, construction of a secreted calcium carbonate test would proceed at a slower rate because calcification, the final stage of chamber formation, would be retarded. If the function of the foraminiferal test were known, then one

might better understand if an efficient calcification system would be of advantage of the organism.

C. Function of the test.

A large body of literature exists dealing with the ecology of recent foraminifers and paleoecology of fossil species, but little or nothing is known of the function of the test. Almost without exception the published observations were made by paleontologists who examined only the empty tests of dead organisms. The "ecology" of foraminifera as interpreted by geologists and paleontologists, in reality concerns itself with the distribution of empty foraminiferal tests. The distribution of tests is more a function of sorting after death rather than a reflection of the distribution of living organisms (R. Wright, unpublished thesis). This accepted interpretation of "ecology" is well exemplified by Phleger's book "Ecology and Distribution of Recent Foraminifera" (1960) which discusses in detail the world-wide distribution of empty foraminiferal tests in recent sediments. The reasons for this unorthodox approach to ecology relate to the history of micropaleontology.

Foraminifers assumed tremendous economic importance with the advent of the search for petroleum. Since that time foraminiferal research has been dominated by several thousand micropaleontologists, mostly engaged in studies for the petroleum industry. As a rule micropaleontologists have strong backgrounds in geology with little or no training in the biological sciences; in fact, very few investigators have ever seen a

living foraminifer. This situation is reflected in the taxonomy of the foraminifera which is based entirely on the morphology of the test with no regard to the living organism. The major taxonomic groups had been established by d'Orbigny (1826) at the time he considered the empty tests to be minute cephalopod shells. Consequently, non-testate foraminifers occasionally found during the course of this investigation are essentially non-classifiable within the group. The present situation is that foraminiferal tests are of little interest to the biologists and are studied almost exclusively by paleontologists. A lack of communication among the two groups has resulted in biological research limited mostly to studies of life cycles, and cytology, and other aspects of foraminiferology which bear little or no relevance to problems in stratigraphy and paleoecology. If the function of the test were understood, then more meaningful paleoecological interpretations of fossil species could be made. An understanding of test function, however, must come through biologically oriented studies of living foraminifers and their relations with the environment.

A consequence of observations of chamber formation in Archias angulatus and several other species has been to acquire a general familiarity with the behaviour of foraminifers under various environmental conditions, both in the field and in the laboratory. These observations in conjunction with scanning electron microscopic investigation of the spatial relationships between the test and the living foraminifer, suggest a general theory of test function (Marszalek, Wright, and Hay, 1969b):

- 1) The most primitive tests are constructed of arenaceous material to

provide weight to counteract buoyancy of the protoplasm. The simplest arenaceous tests seem to serve this and no other function.

2) The test upon elaboration into a tube or series of chambers separated by narrow openings, serves as an effective barrier to retard the effects of unfavorable changes in environmental chemistry.

3) Further specialization may adapt the test for growth under special physical conditions, such as a certain substrate, or for particular symbiotic conditions, such as the greenhouse function of the test of Elphidium (Marszalek, Wright, and Hay, 1969a).

The role of the test as a protective device against predation is not yet understood, but this may account for some specialized forms.

The great variety of test forms in the foraminifers suggests that many taxa are particularly well adapted for specialized ecologic niches. The fact that the foraminifers are among the hardiest of marine protozoa and almost unique in their ability to withstand severe changes in the environment indicates that they have developed a highly efficient means of controlling their immediate environment without encystment or metamorphosis; that means is the test.

The observation by Angell that the cytoplasm in the last one or two chambers of Rosalina is at all times distinct from that which is found in the inner chambers (Angell, 1967b) supports the conclusion that the test in Rosalina as in Archias serves as an environmental buffer. He characterized the cytoplasm of the last chamber as lacking most of the cell organelles found in the inner chambers.

Mature specimens of Archias are characterized by many consecutive chamber rows only a few chambers thick. These younger chambers form an irregular flange around a portion of the earlier formed test. The adaptive significance of the flat test form seems to be to insure maximum light intensity for the zoochlorellae. The volume of the test of mature specimens is greater than is needed to accommodate the cytoplasm and the algal cells; a portion of the test is usually colorless in contrast to the remainder of the test which is occupied by green cytoplasm. It is thought that during favorable environmental conditions the cytoplasm containing the zoochlorellae occupies the flatter portion of the test; during unfavorable conditions, the cytoplasm retracts into the inner more protected portion of the test.

Chamber formation, therefore, is related to growth of the living organism, but in mature specimens it is more directly related to the formation of a protective device against unfavorable changes in the environment.

CONCLUSIONS

The process of chamber formation has been investigated in two foraminifers. Chamber formation in Rosalina floridana as described by Angell (1967b), is representative of the perforate, hyaline foraminifers which form tests of radially oriented calcite crystals. Chamber formation in the imperforate porcelaneous foraminifers which construct tests of randomly oriented calcite crystals is represented by A. angulatus. The modified cytoplasm associated with various stages of the process is very similar in Rosalina and Archias; the mechanics of chamber formation, however, differ significantly and result in two distinct wall structures.

Pseudopods play a major role during chamber formation. In Archias, a free living foraminifer, a characteristic band of short, closely spaced pseudopods supports the chamber during its formation; the completed chamber is not adapted to any particular substrate. No pseudopodial array is formed by Rosalina, a sessile foraminifer; the chamber is formed on the substrate and reflects the topography of that substrate.

Pseudopods are involved in the transport of fibrillar material and other substances which constitute the organic matrix of the chamber wall. Presumably, the final shape of the chamber is controlled by the pseudopods as they migrate over its surface and secrete the organic matrix. The function of the pseudopods during calcification of the organic matrix is not known and could not be deduced from the micrographs.

The process of calcification in Archias shows basic similarities to that observed in other organisms. All biological calcification systems are characterized by a biphasic process. The initial phase is the appearance of an organic matrix. The organic phase, without exception, always forms first and is thought to consist of two components: a) an oriented component usually consisting of complex fibers, and b) an unoriented component consisting of a viscous colloid of several anions. Biochemical analysis of the organic phase shows a protein-carbohydrate combination, occurring in various ratios in different organisms (Moss, 1964). The form of the resulting mineral phase is determined by the organic phase. Some confusion in terminology applied to the organic phase exists in the literature. The true organic membrane associated with the epitaxial growth of oriented calcite crystals as a distinct layer, is confused with the much thicker organic membrane (matrix) associated with the growth of unoriented calcite crystals within a fibrous matrix. The confusion is a result of the usage of identical terms in both light and electron microscopic observations. The relatively thick organic matrix of the chamber wall of Archias and other porcelaneous foraminifers as seen in the electron microscope, is a thin "membrane" in light microscopy and is referred to as such in the literature. The organic control over the mineral phase is evidenced by the micrographs included in this report where the organic matrix is easily seen; in Rosalina the matrix is scanty and is observed only with great difficulty.

The variety of wall structures found in foraminiferal tests suggest that most of the known modes of calcification found among the higher animals, including the deposition of calcium phosphate salts in vertebrate mineralized tissues, have their counterparts in calcite deposition by the foraminifera. It is interesting to note that calcification in R. floridana is similar in some aspects to that which occurs in the nacreous layer of bivalve molluscs (Bevelander and Nakahara, 1968; Towe and Hamilton, 1968), while calcification in A. angulatus is more similar to the mode of mineralization found in the Crustacea and in the vertebrates (Moss, 1964).

Much work is needed before a model for calcification in the foraminifera (and other organisms) can be constructed. This paper indicates some biological and physical aspects of chamber formation and calcification in a protozoan, but it does little to elucidate the means by which calcium ions are concentrated from sea water, combined chemically with carbonate, and deposited as calcite in the form of a test. The observed association of mitochondria and modified cytoplasm involved in chamber formation may be significant. Mitochondria have been implicated in calcium ion storage and transport across membranes (De Luca, Engstrom, and Rasmussen, 1962).

More refined techniques involving autoradiography and biochemical analysis of selected organelles are needed. An Auger electron analysis system soon to be made available (E. Sternglass, personal communication) would allow elemental analysis of thin sections in the scanning electron microscope with resolution of about $100 \text{ \AA}^{\circ}$. With the Auger system, it

will be possible to map ion distribution in cells and in individual organelles in thin section.

REFERENCES

- Angell, R. W. 1967a. The test structure and composition of the foraminifer Rosalina floridana. J. Protozool. 14, pp. 299-307.
- Angell, R. W. 1967b. The process of chamber formation in the foraminifer Rosalina floridana (Cushman). J. Protozool. 14, pp. 566-574.
- Arnold, Z. M. 1964. Biological observations on the foraminifer Spiroloculina hyalina Schulze. Univ. Cal. Publ. Zool. 72, pp. 1-78.
- Bevelander, G., and Nakahara, H. 1969. An electron microscope study of the formation of the nacreous layer in the shell of certain bivalve molluscs. Calc. Tiss. Res. 3, pp. 84-92.
- De Luca, H. F., Engstrom, G. W., and Rasmussen, H. 1962. The action of vitamin D and parathyroid hormone in vitro in calcium uptake and release by kidney mitochondria. Proc. Nat. Acad. Sci. 48, pp. 1604-1609.
- Goreau, T. F., and Goreau, N. I. 1969. The physiology of skeleton formation in corals. II. Calcium deposition by hermatypic corals under various conditions in the reef. Biol. Bull. 117, pp. 239.
- Goreau, T. F., and Goreau, N. I. 1960. Distribution of labeled carbon in reef-building corals with and without zooxanthellae. Sci. 131, pp. 668-669.
- Goreau, T. F. 1961. Problems of growth and calcium deposition in reef corals. Endeavour. 77, pp. 32-39.
- Haller, G. de, Ehret, C. F., and Naef, R. 1961. Technique d'inclusion et d'ultramicrotomie, destinee a l'etude du developpment des organelles dans une cellule isolee. Experientia. 17, pp. 524-529.
- Hedley, R. H. 1964. The biology of foraminifera. Internat. Rev. Gen. Exp. Zool. 1, 1-45.
- Hedley, R. H., Parry, D. M., and Wakefield, J. St. J. 1967. Fine structure of Shepherdella taeniformis (Foraminifera: Protozoa). J. Roy. Micro. Soc. 87, pp. 445-456.

- Jepps, M. W. 1942. Studies on Polystomella Lamarck (Foraminifera). J. Mar. Biol. Assoc. U.K. 25, pp. 607-666.
- Le calvez, J. 1953. Ordre des foraminiferes. In Grasse, P. P., Traite de Zoologie. Masson, Paris. pp. 149-265.
- Lee, J. J., and Zucker, W. 1969. Algal flagellate symbiosis in the foraminifer Archias. J. Protozool., 16, pp. 71-81.
- Loeblich, A. R., and Tappan, H. 1964. Treatise on Invertebrate Paleontology, Part C, Protista 2, Sarcondina, Chiefly "Thecamoebians" and Foraminiferida. Ed. R. C. Moore. Geol. Soc. Amer. & Univ. Kan. Press, Lawrence, Kan. 2 vols., 900 pp.
- Marszalek, D. S., and Hay, W. W. 1968. Marine aquaria for paleontology. J. Geol. Educat. 16, pp. 159-163.
- Marszalek, D. S., and Small, E. B. 1969. Preparation of soft biological materials for scanning electron microscopy. Proceed. 2nd Ann SEM Symposium. ITT-RI, Chicago, Ill. pp. 230-237.
- Marszalek, D.S., Wright, R. C., and Hay, W. W. 1969a. The foraminiferal test as an environmental buffer. Amer. Assoc. Petrol. Geol., Bull. 53, pp. 730.
- Marszalek, D. S., Wright, R. C., and Hay, W. W. 1969b. Function of the test in foraminifera. Gulf Coast Assoc. Geol. Soc., Proceed. (in press in Oct. issue).
- Marszalek, D. S. 1969. Observations on Iridia diaphana, a marine foraminifer. J. Protozool. 16, (in press).
- Moss, M. L., 1964. The phylogeny of mineralized tissues. Internat. Rev. Gen. Exp. Zool. 1, pp. 297-331.
- Myers, E. H. 1940. Observations on the origin and fate of flagellated gametes in multiple tests of Discorbis (Foraminifera). J. Mar. Biol. Assoc. U.K. 24, pp. 201-230.
- Myers, E. H. 1943. Biology, ecology, and morphogenesis of a pelagic foraminifer. Stanford Univ. Pub., ser., Biol. Sci. 9.
- Orbigny, A.D., d'. 1826. Tableau methodique de la classe de Cephalopodes. Ann. Sci. Nat. Paris, ser. 1, pp. 245-314.

- Phleger, F. B. 1960. Ecology and Distribution of Recent Foraminifera. Johns Hopkins Press, Baltimore. viii + 297 pp.
- Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17, pp. 108.
- Small, E. B., and Marszalek, D. S. 1969. Scanning electron microscopy of fixed, frozen, and dried porotozoa. Sci. 163, pp. 1064-1065.
- Towe, K. M., and Hamilton, G. H. 1968. Ultrastructure and inferred calcification in the mature and developing nacre in bivalve molluscs. Calc. Tiss. Res. 1, pp. 306-318.
- Watson, M. 1958. Staining of tissue sections for electron microscopy with heavy metals. J. B. B. C. 3, pp. 475.
- Williamson, W. C. 1858. On the Recent Foraminifera of Great Britain. Ray Soc. Pub., London. xxx + 107 pp.
- Wohlfarth-Bottermann, K. E. 1960. Cytologische Studien VIII. Zum Mechanismus der Cytoplasmas strömung in dünnen Fäden.
- Wright, R. C. 1964. Foraminiferal Ecology in a back reef environment, Molasses Reef, Florida. Thesis (unpublished). Univ. of Illinois. viii + 116 pp.

VITA

Donald Stanley Marszalek was born in Chicago, Illinois on September 23, 1939. He attended primary and secondary schools in Chicago and entered the University of Illinois at Urbana in 1957. After receiving the degree of Bachelor of Science in Geology in 1961 he was with the Department of Paleontology of the University of California at Berkeley for one year of post graduate studies. Several months were spent in Chiapas, Mexico doing geological field work for the University of California. During 1963 through 1965 Mr. Marszalek was in West Africa employed as a field geologist by the Ghana Geological Survey. The Mansi, Fure, and lower Tano river valleys were mapped and prospect drilled for their alluvial gold content. The work was done in conjunction with Bremang Gold Dredge Co., Ltd., Ghana State Mines, under the auspices of the U.S. government. Since 1966 he has been with the Department of Geology at the University of Illinois. During that time several months were spent at the Lerner Marine Laboratory, Bimini, Bahamas and in the Florida Keys mapping recent sediment and organism distributions and studying the organism-sediment relationships. He received the degree of Master of Science in Geology in 1969. Since October, 1969, Mr. Marszalek has been at the Institute of Marine Sciences, University of Miami, Miami, Florida. In September, 1969, he was appointed Research Scientist at the Institute of Marine Sciences, in the Division of Marine Geology and Geophysics and the Division of Functional Biology. His publications include "Marine

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ASPECTS OF CHAMBER FORMATION BY ARCHIAS



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